In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology & Therapeutics
The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3
Date 25-July-88
Sensory transmission was studied in trigeminal root ganglia (TRG) of guinea pigs, using intracellular recording techniques. One approach was to examine in detail the effects of applications of different $K^+$-channel blockers on the membrane voltage responses and outward currents of TRG neurons, in order to better understand the fundamental processes that affect their excitabilities and repetitive spike discharge. The second approach was to examine several endogenous substances for their effects on the excitabilities of TRG neurons.

In addition, a strategy was developed for electrophysiological recording from neurons in human sympathetic ganglia. Successful investigations of these neurons revealed properties similar to certain reported characteristics of sympathetic neurons in experimental animals, including high (~29 MΩ) input resistances, pharmacological sensitivity of spikes to the specific Na$^+$-channel blocker tetrodotoxin (TTX, 1 μM) and to selective $K^+$-channel blockers -- 4-aminopyridine (4-AP, 1 mM) and tetraethylammonium (TEA, 10 mM). The investigations demonstrated the potential value of these in vitro preparations for studies of the human condition.

The investigations in TRG neurons demonstrated that bath applications of TEA (0.1-10 mM) and 4-AP (0.05-5 mM) or Cs$^+$ applied internally from the recording electrode, produced an increase in input resistance and a decrease threshold for spike generation in all neurons. Also, applications of 4-AP increased subthreshold oscillations of the membrane potential and enhanced the repetitive spike firing evoked by intracellular injections of current pulses, or elicited spontaneous firing. In contrast, TEA or Cs$^+$ applications blocked the oscillations and the spike afterhyperpolarizations (AHPs) without exaggerating repetitive discharge. These investigations suggested
that several pharmacologically distinct $K^+$-currents contribute to the control of excitability in TRG neurons. Comparison of combined actions of 4-AP and TEA with those of $Cs^+$, suggested that other ions in addition to $K^+$ may contribute to postspike events.

Single electrode voltage-clamp analyses revealed transient outward currents that were evoked at the termination of hyperpolarizing voltage commands from holding potentials near -40 mV. The activation was rapid (<5ms) and inactivation ($\tau$=19 ms) complete at potentials within the activation range (-40 to -75 mV). During combined application of TTX (1 μM) and TEA (10 mM), fast activating, sustained currents (>1 s) were evoked by depolarizing commands from holding potentials near -70 mV. These currents were blocked completely by the additional applications of 4-AP (5 mM).

Applications of TEA (0.1 mM to 10 mM) produced dose-dependent reductions of the transient outward currents. Applications of $Cs^+$ also blocked the currents. However, administrations of 4-AP (0.05 to 5 mM) only slightly reduced these currents and high doses of muscarinic agonists had no effect. The high sensitivity to TEA, and not to 4-AP, suggest a fundamental distinction from similar currents observed previously in other neurons of vertebrates and invertebrates, and hence this transient outward current in TRG neurons, is termed $I_{(T)}$.

The kinetics of $I_{(T)}$ suggest its involvement in the spike AHPs. Therefore, blockade of $I_{(T)}$ by TEA may interfere indirectly with the re-activation of voltage-dependent $Na^+$-channels, leading to decreases in repetitive discharge ability. The TEA-insensitive sustained outward current presumably has an inhibiting influence on repetitive discharge. Conditions that interfere with this current, such as blockade of $K^+$-channels by 4-AP without a significant blockade of $I_{(T)}$, strongly favour the generation of
repetitive discharge in TRG neurons.

The investigations using electrical stimulation of axons revealed that changes in the resting potential could inhibit the invasion of spikes into the perikarya, or facilitate the generation of ectopic spike discharges. Applications of 4-AP (1 mM) facilitated the perikaryal invasion of spikes evoked by axonal stimulation, and also resulted in the appearance of fast (~10 ms) depolarizations that reached spike threshold in the absence of applied stimuli. These investigations provided direct evidence that the perikarya of sensory neurons are capable of spike generation, and suggest that this behavior may occur in normal or pathophysiological conditions.

The most notable effects of autacoids were those of substance P and histamine, whereas bradykinin did not affect neuronal membrane properties. Applications of substance P in micromolar doses evoked large (up to 45 mV), reversible depolarizations in the majority of neurons, whereas histamine applications produced similar depolarizations only in a small portion of the TRG neurons. Increases in the repetitive discharge abilities of neurons were evident during substance P-induced depolarizations. Studies on the ionic mechanism of substance P action revealed that the peptide-applications resulted in activation of inward currents as well as blockade of outward currents. In addition, it was shown that Na⁺ and Mg²⁺ were involved in the mechanism of action.

These findings represent the first demonstration of the profound actions of substance P on the perikaryal membranes of sensory neurons in mammals. The excitatory actions of this endogenous peptide also give rise to the possibility of physiological actions of substance P at multiple sites in the trigeminal system.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 General introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Anatomical organization of the trigeminal root ganglion and other sensory ganglia</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Embryology and morphology of neurons in sensory ganglia</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Involvement of perikarya in sensory transmission</td>
<td>5</td>
</tr>
<tr>
<td>1.4.1 Role of satellite cells</td>
<td>7</td>
</tr>
<tr>
<td>1.4.2 Variability of neuronal populations in sensory ganglia</td>
<td>7</td>
</tr>
<tr>
<td>1.5 Sensory modalities transmitted by primary afferent fibers</td>
<td>8</td>
</tr>
<tr>
<td>1.5.1 Relationship of afferent fiber diameters to sensory modalities</td>
<td>9</td>
</tr>
<tr>
<td>1.5.2 Morphology of peripheral terminations of primary afferent fibers in relation to sensory modalities</td>
<td>9</td>
</tr>
<tr>
<td>1.6 Peripheral neural mechanisms of pain</td>
<td>10</td>
</tr>
<tr>
<td>1.6.1 Triple response</td>
<td>11</td>
</tr>
<tr>
<td>1.6.2 Axon reflex</td>
<td>11</td>
</tr>
<tr>
<td>1.6.3 Autonomic reflexes</td>
<td>12</td>
</tr>
<tr>
<td>1.7 Endogenous algogenic substances</td>
<td>12</td>
</tr>
<tr>
<td>1.7.1 Potassium</td>
<td>12</td>
</tr>
<tr>
<td>1.7.2 Changes in extracellular pH</td>
<td>13</td>
</tr>
<tr>
<td>1.7.3 Acetylcholine</td>
<td>13</td>
</tr>
<tr>
<td>1.7.4 5-Hydroxytryptamine</td>
<td>14</td>
</tr>
<tr>
<td>1.7.5 Histamine</td>
<td>14</td>
</tr>
<tr>
<td>1.7.6 Bradykinin</td>
<td>14</td>
</tr>
<tr>
<td>1.7.7 Substance P</td>
<td>15</td>
</tr>
<tr>
<td>1.8 Substance P as neurotransmitter of primary afferent neurons</td>
<td>16</td>
</tr>
<tr>
<td>1.9 Membrane electrical properties of primary sensory neurons</td>
<td>18</td>
</tr>
<tr>
<td>1.9.1 Control of membrane excitability</td>
<td>19</td>
</tr>
<tr>
<td>2 METHODS</td>
<td>22</td>
</tr>
<tr>
<td>2.1 Animals</td>
<td>22</td>
</tr>
<tr>
<td>2.1.1 Source</td>
<td>22</td>
</tr>
<tr>
<td>2.1.2 Animal feed and housing</td>
<td>22</td>
</tr>
<tr>
<td>2.2 Surgical procedures for removal of the TRG</td>
<td>22</td>
</tr>
<tr>
<td>2.3 Procedure for obtaining human trigeminal root ganglia at autopsy</td>
<td>24</td>
</tr>
</tbody>
</table>
2.4 Procedure for obtaining human sympathetic ganglia ........................................... 24
2.5 Electrophysiological recordings ........................................................................... 26
  2.5.1 Ion substitutions ............................................................................................... 28
  2.5.2 Drugs ................................................................................................................ 28
  2.5.3 Electrodes ............................................................................................................ 28
  2.5.4 Recording equipment ........................................................................................ 29

3 RESULTS .................................................................................................................... 30
  3.1 Dependence of resting membrane potential 
on extracellular cations ......................................................................................... 30
    3.1.1 Changes in extracellular [K⁺] ............................................................................ 30
    3.1.2 Changes in extracellular [Na⁺] ......................................................................... 32
    3.1.3 Changes in extracellular [Ca²⁺] and Co²⁺-application ................................... 32
  3.2 Membrane properties of TRG neurons ................................................................. 35
    3.2.1 Membrane potential oscillations ..................................................................... 35
    3.2.2 Action potentials and afterhyperpolarizations .............................................. 35
    3.2.3 Effects of ionic channel blockade on spikes ................................................ 37
  3.3 Membrane properties of human sympathetic neurons .......................................... 39
  3.4 Effects of K⁺-channel blockers ............................................................................. 42
    3.4.1 Current clamp: effects of tetraethylammonium 
on subthreshold potentials ......................................................................................... 42
    3.4.2 Effects of TEA on spikes and repetitive discharge ...................................... 46
    3.4.3 Effects of 4-aminopyridine on subthreshold 
potentials of TRG neurons .................................................................................. 50
    3.4.4 Effects of 4-AP on spikes and repetitive discharge ..................................... 50
    3.4.5 Combined applications of 4-AP and TEA ...................................................... 53
    3.4.6 Effects of intracellular Cs⁺ ............................................................................. 55
  3.5 Voltage-clamp analysis .......................................................................................... 59
    3.5.1 Transient outward currents ............................................................................. 59
    3.5.2 Effects of ionic substitution .............................................................................. 60
    3.5.3 Ineffectiveness of muscarinic agents ............................................................... 64
    3.5.4 Effects of 4-AP and TEA ................................................................................. 64
    3.5.5 Effects of internal Cs⁺ ....................................................................................... 68
    3.5.6 Other outward currents ................................................................................... 68
    3.5.7 Combined applications of 4-AP and TEA ........................................................ 72
  3.6 Studies on the perikaryal invasion of spikes in the TRG ...................................... 76
    3.6.1 Changes in resting membrane potential ......................................................... 76
    3.6.2 Effects of 4-AP ................................................................................................. 79
  3.7 Studies of autacoid effects in TRG neurons ............................................................ 83
    3.7.1 Bradykinin ....................................................................................................... 83
    3.7.2 Histamine ....................................................................................................... 83
3.7.3 Substance P ................................................................. 83
3.7.3.1 Effects on subthreshold membrane properties ............... 83
3.7.3.2 Effects of substance P on spikes and repetitive discharge .................................................. 87

3.8 Studies on the ionic mechanism of substance P actions ......... 87
3.8.1 Effects of changes in extracellular [Na\(^+\)] ......................... 87
3.8.2 Effects of changes in extracellular [Mg\(^{2+}\)] ..................... 91
3.8.3 Effects of Ca\(^{2+}\)-channel blockade .............................. 91
3.8.4 Voltage-clamp studies of substance P actions in the presence of K\(^+\)-channel blockers ................. 94

4 DISCUSSION ................................................................. 97

4.1 Membrane potential dependence on extracellular cations .......... 100
4.2 Electrical membrane properties of TRG neurons .................. 100
4.2.1 Subthreshold responses ............................................... 102
4.2.2 Action potentials .................................................... 101
4.2.3 Postspike afterhyperpolarizations ................................. 102

4.3 Membrane electrical responses of human sympathetic neurons .. 103

4.4 Differences in the actions of K\(^+\)-channel blockers on TRG neurons .................................................. 105
4.4.1 Membrane potential oscillations ................................... 106
4.4.2 Repetitive spike firing ............................................... 108

4.5 Comparison of transient outward current [I(T)] with I\(_A\) ....... 109
4.5.1 Ionic species mediating I(T) ........................................ 110
4.5.2 Membrane repolarization ............................................ 111
4.5.3 Other outward currents ............................................... 112
4.5.4 Significance .......................................................... 113
4.5.5 Bursts and ionic mechanisms ...................................... 114
4.5.6 Significance .......................................................... 115

4.6 Spike initiation in the TRG ............................................ 116

4.7 Membrane responses to autacoids .................................... 117

4.8 Ionic mechanism of substance P action ............................. 118
4.8.1 Implications for sensory transmission ............................ 121

4.9 Directions for future research ....................................... 123

5 CONCLUSIONS ............................................................ 126

6 REFERENCES ............................................................... 130
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I. Summary of spike and afterhyperpolarization (AHP) characteristics</td>
<td>38</td>
</tr>
<tr>
<td>Table II. Effects of tetraethylammonium (TEA, 10 mM) on electrical properties</td>
<td>45</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematic diagram of the transport chamber</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Schematic representation of the recording chamber</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Dependence of membrane potential on extracellular [K+]</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>Effects of Na⁺-deficient solutions</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>Two types of action potentials in TRG neurons</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>Responses of human neurons</td>
<td>41</td>
</tr>
<tr>
<td>7</td>
<td>Effects of combined TTX and TEA applications on spikes and subthreshold oscillations</td>
<td>44</td>
</tr>
<tr>
<td>8</td>
<td>Comparison of TEA and 4-AP effects on spikes and subthreshold oscillations</td>
<td>48</td>
</tr>
<tr>
<td>9</td>
<td>Effects of TEA and 4-AP on spikes in a human neuron</td>
<td>49</td>
</tr>
<tr>
<td>10</td>
<td>K⁺-channel blockade and burst activity</td>
<td>52</td>
</tr>
<tr>
<td>11</td>
<td>Effects of Ca²⁺-channel blockade on spikes</td>
<td>54</td>
</tr>
<tr>
<td>12</td>
<td>Effects of Cs⁺ on spikes</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td>Spike afterpotentials in TRG neurons</td>
<td>58</td>
</tr>
<tr>
<td>14</td>
<td>Transient outward currents in TRG neurons</td>
<td>62</td>
</tr>
<tr>
<td>15</td>
<td>Transient outward currents in human neurons</td>
<td>63</td>
</tr>
<tr>
<td>16</td>
<td>Effects of 4-AP and TEA</td>
<td>66</td>
</tr>
<tr>
<td>17</td>
<td>Dose-response of K⁺-channel blockers</td>
<td>67</td>
</tr>
<tr>
<td>18</td>
<td>Effects of Cs⁺ on spikes and transient outward currents</td>
<td>69</td>
</tr>
<tr>
<td>19</td>
<td>Voltage-clamp responses to depolarizing command steps</td>
<td>71</td>
</tr>
<tr>
<td>20</td>
<td>Effects of K⁺-channel blockers on other outward currents</td>
<td>74</td>
</tr>
<tr>
<td>21</td>
<td>4-AP-sensitive outward current</td>
<td>75</td>
</tr>
<tr>
<td>22</td>
<td>Comparison of responses to axonal and perikaryal stimulation</td>
<td>78</td>
</tr>
</tbody>
</table>
23. Effects of changes in resting potential on spike invasion......
24. Effects of 4-AP on spike invasion..........................
25. Effects of histamine on a TRG neuron.....................
26. Depolarizing responses to substance P.....................
27. Facilitation of repetitive discharge......................
28. Reduction of responses in Na\(^+\)-deficient perfusates.....
29. Reduction of responses in Mg\(^{2+}\)-deficient perfusates...
30. Effects of substance P during K\(^+\)-channel blockade.......

- x -
ACKNOWLEDGEMENTS

I wish to express my gratitude to Professor Ernest Puil without whose support and encouragement this work would not have been possible. I would also like to thank other members of the Department of Pharmacology & Therapeutics for their patience with me these last few years. Special thanks are reserved for Drs. B. R. Sastry and D. M. J. Quastel for their helpful discussions of my work.

The financial support of the Canadian Heart Foundation is gratefully acknowledged.
This thesis is dedicated to my parents --

tamara and George Spigelman

who constantly struggle to improve my well-being.

"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes."

Marcel Proust
1 INTRODUCTION

1.1 General introduction

Physiological and pharmacological investigations of the membrane properties of sensory neurons are important to an understanding of peripheral nervous system (PNS) function. Transmission of sensory signals from the peripheral receptors is mediated by ionic mechanisms in the neuronal membrane. Alterations in the electrical properties of the membrane can profoundly influence impulse propagation along the primary afferent fibers to the central nervous system (CNS). Endogenous substances and therapeutic agents may act through ionic mechanisms in the membrane, thereby influencing the neuronal excitabilities. In addition, certain pathophysiological conditions in humans involve perturbations of neuronal membrane function. Hence, investigations of drug-induced alterations in membrane properties are prerequisite to an understanding of PNS function from a clinical standpoint.

Although neurons of the dorsal root ganglion (DRG) have been subjected to extensive investigations during the past century, neurons of the trigeminal root ganglion (TRG), especially their membrane properties, have not been studied to the same extent. This oversight is due partly to the generalized assumption that the membrane properties of TRG neurons are likely similar to their counterparts in the DRG. In addition, the cell bodies of these neurons are believed to participate only secondarily (i.e., in a supportive role) in the transmission of sensory information along their primary afferent axons. The assumption does not have a firm basis because the role of neuronal cell bodies in this transmission has not been thoroughly examined with electrophysiological methods, particularly with intracellular recording techniques.
The investigations described in this thesis focus attention on the membrane electrical properties using these techniques and examine certain mechanisms by which sensory impulse transmission can be modulated in the TRG neurons. For a fuller comprehension of the experimental design, the anatomical organization and involvement of the ganglia in sensory transmission will be reviewed. Secondly, it may be instructive to summarize, succinctly, the peripheral mechanisms of sensation, including endogenous agents that may mediate nociception.

1.2 Anatomical organization of the trigeminal root ganglion and other sensory ganglia

The dorsal root ganglia and cranial sensory ganglia such as the trigeminal root ganglion are collectively referred to as craniospinal, cerebrospinal or sensory ganglia. Sensory ganglia form swellings on the dorsal roots or cranial sensory nerves near their points of entry into the CNS. Three exceptions to this arrangement are the primary sensory neurons of the visual and olfactory systems and the mesencephalic nucleus of the trigeminal nerve which lies within the brainstem. Sensory ganglia contain the cell bodies (perikarya) of primary afferent neurons and the proximal portions of their axons. The ganglia also contain the Schwann and satellite cells that form sheaths which are associated with the axons and the perikarya, respectively, of the neurons. In addition, the supportive elements of the peripheral nervous system also are present: these include the connective and vascular tissues.

In mammals, the TRG is the largest sensory ganglion in the body and is composed of three divisions -- ophthalmic, maxillary and mandibular. Each ganglion is formed at the point of convergence of its three divisions on the
floor of the cranial vault, with the largest (mandibular) branch in the most lateral position. The bilateral branches provide innervation to the mucosal lining of oral cavities, the skin, and the muscles of mastication (the latter are innervated by neuronal perikarya within the trigeminal mesencephalic nucleus in the brainstem). Primary afferents in the trigeminal nerve innervate the cornea (McNaughton, 1938) as well as blood vessels in the circle of Willis (Mayberg et al., 1981; Liu-Chen et al., 1984), one of the few nociceptive areas within the cranium (Wolff, 1963). The mystacial vibrissae (whiskers) possessed by most mammals are served by fibers of TRG neurons. Also, sensory innervation of the tooth pulp, surrounding gingiva and periodontal membrane is supplied by the trigeminal nerve (Kelly, 1981).

Sensory ganglia are surrounded by a multilayered perineurial sheath and a fibrous outer epineurium that are very similar to those of peripheral nerve trunks (Lieberman, 1976). Since the cranial and sensory nerve roots perforate the inner meningeal layer of the dura mater, they are bathed in cerebrospinal fluid (Gamble, 1976). However, the ganglia possess a rich supply of blood vessels that do not share the protective mechanisms present in the brain; the contents of sensory ganglia are exposed to the ions and molecules that pass across the capillary walls. Compared with spinal ganglia, the blood vessels of the TRG appear to be less permeable to the intravenous injection of protein tracers (Arvidsson, 1973). This may account for the relative immunity of the ganglion to many toxins and drugs that can affect peripheral nerves (Selby, 1984).

1.3 Embryology and morphology of neurons in sensory ganglia

During the course of development, cells of the neural crest migrate into clumps along the neural tube forming the primordia of the spinal ganglia
Formation of the cranial ganglia is more complicated, because unlike their spinal counterparts, cranial ganglia originate from the neural crest cells as well as from the cells of the ectodermal placodes (Weston, 1970). The relative contribution of these two sources to the ganglion cells is specific to each particular ganglion. Thus, the TRG receives contributions from the neural crest cells and placodal ectoderm, whereas the petroossal and nodose ganglia are derived predominantly from the ectodermal placodes (Johnston and Hazelton, 1972).

The undifferentiated ganglion cells send out several small pseudopodia but during further development these are reduced to two processes, one at each pole of the cell. The processes become oriented such that one is directed towards the CNS and the other towards the periphery (Krieg, 1966). The peripheral process is usually thicker and grows at a faster pace (Pannese, 1974). With continued growth the perikaryon begins to move away from the aligned processes but remains connected by a stem process. This differentiation results in the formation of a T-junction at the point where the stem process (initial segment or "connecting piece" of Cajal, 1907) bifurcates into the central and peripheral processes.

In mammals, especially carnivores, the stem process becomes convoluted, sometimes with complete envelopment of its perikaryon (Svaetichin, 1951; Scharf, 1958). This development of a glomerulus takes place only late during the first few postnatal weeks (Cajal, 1909). The exact significance of the glomeruli is not known, but they may confer a delay in the invasion of the perikarya by impulses from the periphery (cf. Dun, 1955). Sometimes, at the point where the unmyelinated stem process emerges from its glomerular form, a myelin sheath may be acquired. This internodal myelin is usually thinner than that of the dorsal root fibers (Rexed and Sourander, 1949).
Myelinated stem processes bifurcate at the nodes of Ranvier and are normally constricted at these points, whereas the T-junctions of unmyelinated processes are usually enlarged (Ha, 1970).

1.4 Involvement of perikarya in sensory transmission

The offstream position of the perikarya of sensory ganglia with respect to their axonal processes that transmit information from the periphery to the CNS has led to the view that the perikarya are of limited significance in terms of an electrophysiological role in the intact animal (Lieberman, 1976). This view is supported by the evidence that the majority of neuronal perikarya in the ganglia appear to be devoid of synaptic contacts. However, a very small number of synaptic bouton terminations have been demonstrated conclusively to impinge on perikarya in the cat DRG. The axons that form these contacts originate in the spinal cord (Kayahara et al., 1984).

Spontaneous electrical activity is not a feature of primary sensory neurons in the in vitro preparations, prior to passage of current through the recording electrode or on electrical stimulation of the axonal processes (cf. Puil et al., 1986; Puil and Spigelman, 1988). Furthermore, Darian-Smith and colleagues (1965) showed that orthodromic impulses initiated in a large myelinated nerve fiber will invade the central terminations before excitation of the perikaryon is observed extracellularly in the TRG.

Although some available evidence indicates that synaptic activity and action potentials in TRG perikarya do not contribute significantly to ongoing electrical activity along the afferent fibers, certain biochemical and electrophysiological observations suggest an active participation of the perikarya in trigeminal afferent transmission. The neurons synthesize compounds necessary for the activities of their central and peripheral processes. Substances synthesized within the perikarya travel by fast and
slow axoplasmic transport to both (terminal) ends where presumably they are utilized for various functions (e.g. transmitter release). The synthesizing or "factory" role has been localized to the perikarya by the biochemical and histochemical demonstrations of putative transmitters such as S-glutamate and substance P within primary afferent neurons, including those of the TRG (Hökfelt et al., 1975).

Electrophysiological evidence from studies on DRG neurons also supports a more active role of perikarya in afferent transmission. Thus, Tagini and Camino (1973) have found that the repeated activation of large diameter neurons by electrical stimulation of the peripheral nerve evokes spikes that travel towards the ganglion where additional action potentials are generated in the vicinity of the perikaryon. Curiously, these "anomalous" action potentials always seemed to propagate in the antidromic direction towards the periphery, but not to the central terminations.

Kirk (1974) recorded spontaneous impulses that periodically discharged from the dorsal rootlets of ganglia that were isolated from the periphery by transection of the spinal nerve. In contrast to the above observations of Tagini and Camino (1973) these spontaneous impulses traveled only orthodromically from the ganglion towards the spinal cord, and not towards the sectioned nerve. Such ectopic discharges have been suggested to be the source of abnormal sensations which occur following amputation (Carlen et al., 1978).

Recent investigations of TRG neurons in vivo, using intracellular recording techniques, have revealed that the perikaryal membranes of TRG neurons may act as a filter of inputted signals (Puil et al., 1986, 1987). The ability of TRG neurons to either amplify or dampen signals, depending on the input frequencies, may provide an explanation for the abilities of
sensory neurons to generate "new action potentials" in response to excitation of their axons.

1.4.1 Role of satellite cells. In the course of embryological development each neuronal perikaryon becomes enveloped by a capsule formed by small flattened cells, i.e., satellite cells (Cajal, 1909). Like Schwann cells of peripheral nerve fibers, these probably derive from the neural crest (cf. Weston, 1970). Satellite cells are closely apposed to one another and to the neuronal perikarya which they always envelop (Lieberman, 1976). Gap junctions between satellite cells narrow the intercellular spaces even more (Pannese, 1969). However, large molecules still can permeate freely to neuronal surfaces (Rosenbluth and Wissig, 1964).

Although the cytology of satellite cells is well established, the functions of satellite cells and their interaction with neurons are virtually unknown. Nevertheless, one role of satellite cells has been ascertained in the glutamine-glutamate cycle in which satellite cells take up glutamate and transform it to glutamine which is subsequently released (Schon and Kelly, 1974). The glutamine may be taken up by neurons to provide a substrate for synthesis of glutamate and its subsequent utilization in various ways.

1.4.2 Variability of neuronal populations in sensory ganglia. The perikaryal diameters of neurons in sensory ganglia differ considerably. For example, neuronal diameters have been described in the range of 15-110 μm within the human sensory ganglia (Ohta et al., 1974). We have observed perikaryal diameters of 100 μm in trigeminal root ganglia obtained 4 hrs postmortem from an adult human male (Spigelman and Puil, unpublished observations).

Histological studies of sensory neurons revealed separate populations of large light and small dark cells (Andres, 1961; Scharf, 1958). In the
trigeminal and spinal ganglia of rodents these become apparent only post-
natally (Kalina and Wolman, 1970). In many studies, consistent differences
have been observed in ultrastructural features of the two neuronal classes
(Lieberman, 1976). These differences have yet to be reconciled with the
possible functional differences of light and dark neurons. If size alone is
considered, the small dark cells are more likely associated with small
diameter axons and therefore likely to transmit sensory information specific
to these fibers (cf. Gobel, 1974; Ohnishi and Dyck, 1974).

1.5 Sensory modalities transmitted by primary afferent fibers

The somatic sensory system of mammals is capable of conveying five basic
qualities of sensations that can be evoked by stimulation of various tissues
in the body. Touch, warmth, cold and pain are best developed in the skin,
but also can be present in other body parts including visceral organs. Each
modality is served by a specific set of primary afferent fibers. Acting
alone each nerve fiber will only evoke a single primary sense quality.
However, more complex sensations are produced within the CNS from the
activity of different sets of nerve fibers. The fifth sensation concerns
the position and movement of the joints (kinesthesia) that depends on the
activity of primary afferent fibers which innervate ligaments and capsules
of joints. In addition, primary afferents innervating skeletal muscles may
also contribute to kinesthesia.

Thus, single qualities of sensation can be identified in humans when
stimulated appropriately, although most conscious sensations evidently arise
from the central synthesis of sensory input through the various sets of
afferent fibers. Whereas only one elementary sensory experience can be
elicited by an appropriate stimulus, changes in the strength or temporal
characteristics of the stimulus can derive a variation of the sensory modality. For example, a submodality such as itch can be converted to pain by increasing intensity, or touch can be converted to flutter by increasing the frequency of a stimulus (Bishop, 1946).

1.5.1 Relationship of afferent fiber diameters to sensory modalities. In the course of investigations on peripheral sensory mechanisms a correlation was observed between the various modalities of sensation and the diameters of primary afferent fibers that mediate these sensations. In the current classification (Mountcastle, 1980) the large myelinated fibers (Aβ: 6-12 μm in diameter, 35-75 m/s conduction velocity) serve a number of sensations that are grouped together as mechanoreceptive. These include touch-pressure, flutter-vibration and position sensibility. The small myelinated fibers (Aδ: 1-5 μm, 5-30 m/s) and the unmyelinated fibers (C: 0.2-1.5 μm, 0.2-2 m/s) that innervate the furry skin of many mammals, such as rodents, provide the crude sense of contact. However, mechanoreception in the skin of the primate hand is almost entirely dependent on the innervation by larger myelinated fibers (Perl, 1968). Pain induced by pinpricks and cold sensation also are mediated by fibers in the Aδ range, whereas sensations of burning pain and warmth are served by the C-fibers (Zotterman, 1933; Bishop and Heinbecker, 1935; Clark et al., 1935; Bessou and Perl, 1969).

1.5.2 Morphology of peripheral terminations of primary afferent fibers in relation to sensory modalities. The many varieties of sensory endings found in the skin and visceral tissues of mammals can be classified into broad groups: (1) free (bare) nerve endings, (2) endings with expanded tips and (3) encapsulated endings (Miller et al., 1960). The free nerve endings are densely distributed throughout all layers of the skin. Both the Aδ and
C-fibers terminate in free endings which are appropriate for fibers that mediate the more "primitive" sensations such as pain. Endings with expanded tips (Merkel's tactile disks) are those associated with specialized epidermal cells. As in the case of free nerve endings, a single nerve fiber supplies many tactile disks (Merzenich and Harrington, 1969).

Encapsulated nerve endings are formed around the terminals of large myelinated nerve fibers. The different types of end organs seem to predestine the dynamic sensitivities of mechanoreceptive nerve endings. For example, Meissner afferents are velocity detectors that discharge brief bursts of impulses which decline rapidly (adapt) following a step indentation of the skin, and recur briefly on removal of the stimulus. Such endings are sensitive selectively to low-frequency stimuli in the 30-40 Hz range and are thought to serve the mechanoreceptive submodality of flutter. In contrast, Pacinian corpuscles which provide the submodality of vibration may function optimally at stimulus frequencies of 250-300 Hz (Merzenich and Harrington, 1969).

1.6 Peripheral neural mechanisms of pain

It is known from animal experimentation as well as from stimulation of human subjects that impulses initiated by cutaneous painful stimuli are propagated via the Aδ and C-fibers. Both fiber groups are considered polymodal because they are capable of responding to different forms of destructive energy.

It is not known how the transduction of painful stimuli to impulses takes place at the peripheral terminals of nociceptive afferents. One possibility is that free nerve endings are activated directly by noxious stimuli in a manner analogous to that observed in encapsulated endings of
mechanoreceptive afferents. Another suggestion involves the release of pain producing substances (algogens) either from damaged tissue or from the nerve terminals themselves. These algogens in turn act as agonists on receptors located on the free nerve endings, leading to excitation of primary afferent fibers.

1.6.1 Triple response. This phenomenon is initially apparent as sudden and intense pain subsequent to a damaging stimulus to the skin. An unpleasant state of low intensity, poorly localized pain usually follows. A pronounced vasodilation in the injured area leads to the formation of a wheal which is soon surrounded by a wider area of less intense vasodilation of the skin. Local reddening, wheal formation and flushing surround of the skin comprise the sequence described by Lewis (1942) as the triple response. The reduction in threshold for pain (hyperalgesia) in the injured area and in part of the surrounding flare may persist for days. The secondary hyperalgesia in a wide region outside the flare may last for several hours after the initiating stimulus. The two hyperalgesias may have different causal mechanisms (Mountcastle, 1980).

1.6.2 Axon reflex. The flare formation that surrounds the locally injured site can be produced only when the peripheral axons are intact. Sectioning the central portion of the dorsal roots does not lead to degeneration of peripheral axons, and the flare formation is preserved (Chapman et al., 1961). In contrast, degeneration of peripheral axons does not deter local vasodilation and wheal formation, whereas the surrounding flare is lost. These observations have led to the conclusion that the cutaneous vasodilation which is apparent as a flare is a result of antidromic activity in the peripheral branches of nociceptive afferents.
Vasodilation occurs concomitantly with plasma extravasation and the two processes have been termed neurogenic inflammation (Jancsó et al, 1967).

1.6.3 Autonomic reflexes. Noxious stimuli can evoke reflex reactions mediated by the autonomic nervous system as part of the defensive or aggressive response of the organism (Mountcastle, 1980). Some of these reactions arise as a result of synaptic activation of neurons in the CNS, or the antidromic activation of nociceptive afferents. Impingements of the collaterals of peripheral sensory fibers on postganglionic sympathetic neurons have been observed; these can be excited by afferent stimulation (cf. Jessell, 1983).

1.7 Endogenous algogenic substances

Extracts from damaged tissue can cause intense pain when injected into skin (Mountcastle, 1980). This observation led to nearly 60 years of research on the endogenous algogen that may be released from the tissue by a damaging stimulus, leading to activation of nociceptive afferents. Several substances that may be present in extracts of damaged tissue are known to cause pain and each has been proposed as the chemical mediator of pain.

1.7.1 Potassium. $K^+$ administered as a salt solution by intradermal, intramuscular, intraarterial or intravenous injections produces pain (Keele and Armstrong, 1964). In contrast to other pain-producing substances that may require a specific membrane receptor, an increase in extracellular $[K^+]$ will cause neuronal depolarization simply through a change in $[K^+]$ gradient between the extracellular and intracellular compartments. A rise in extracellular $[K^+]$ elicits pain that has a more rapid onset and briefer duration than pain produced by extracts of damaged tissue, such as blister
fluid (Keele and Armstrong, 1964). Therefore $K^+$ is unlikely to be the sole or main agent acting peripherally to evoke painful sensations following injury to tissue.

1.7.2 Changes in extracellular pH. Low pH or alkaline solutions also cause pain on application to a blister base, but such changes are not normally associated with the injuries produced by a pinprick or a razor cut. Also, analgesia follows the initial painful effects of applications of acidic solutions. Changes in extracellular pH can elicit responses in the perikarya of sensory neurons (Gruol et al., 1980; Krishtal and Pidoplichko, 1981). For example, a rapid decrease in the extracellular pH can evoke an inward current that is carried by $Na^+$ and $K^+$ ions (Krishtal and Pidoplichko, 1981). These authors observed that a greater number of TRG, than DRG, neurons were capable of responding to pH changes, although the significance of this finding remains obscure. However, the sensory endings of these neurons also may be endowed with receptors for protons and serve as sensors of $[H^+]$ in various regions of the body (Krishtal and Pidoplichko, 1981).

1.7.3 Acetylcholine. Subcutaneous or intravascular injections of acetylcholine (ACh) as well as its applications to blister base produce pain (Keele and Armstrong, 1964). The onset of pain is fast and a refractoriness that develops to subsequent applications of ACh is very pronounced. Nicotinic receptors may be involved in the production of pain by ACh, because applications of muscarinic agonists such as methacholine or pilocarpine are not painful, whereas d-tubocurarine or hexamethonium antagonize the pain induced by ACh. The sensory afferent fibers that are excited by Ach belong both to the myelinated and unmyelinated categories and include fibers that respond to pressure (Fjällbrandt and Iggo, 1961). The relatively high
concentrations of ACh that are needed to elicit pain on subcutaneous injection are not likely to be released by tissue damage even in the presence of anticholinesterases (Hurley and Koelle, 1958). Hence, it is unlikely that ACh is a prime candidate for the pain-producing substance.

1.7.4 5-hydroxytryptamine. The pain produced by 5-hydroxytryptamine (5-HT) on application to the blister base has a delayed onset and much longer duration than the pain caused by ACh application (Keele and Armstrong, 1964). Close arterial injections of 5-HT into an innervated area of the skin produce a long-lasting discharge of impulses along cutaneous sensory fibers (Fjällbrandt and Iggo, 1961; van Gelder, 1962). This component of serum platelets is liberated during clotting (Janeway et al., 1918) and causes vasoconstriction (Mills et al., 1968). Mast cells in the trigeminal ganglia of rats and guinea pigs contain 5-HT (Lehtosalo, 1984), but their exact physiological role in the ganglia has not been identified.

1.7.5 Histamine. The role of this particular autacoid in the vascular responses to various noxious stimuli has been established largely by the early work of Lewis and Grant (1924) and Lewis (1927). The response to histamine has been associated closely with the name triple response. Lewis (1942) suggested that histamine produced itch but not pain. However, other investigators have demonstrated the algogenic actions of histamine on application to abraded skin or by intradermal injections (Rosenthal and Minard, 1939).

1.7.6 Bradykinin. This nonapeptide was among the first substances shown to be present in exudates from painful tissue (Chapman et al., 1961; Keele and Armstrong, 1964). Although bradykinin is a good candidate for the endogenous algogen, several observations are at odds with this proposal. First, appearance of this autacoid in blister fluid collections is only
detected after contact with glass, which activates the production of kinins (Argent et al., 1954). Second, a complete destruction of afferent nerve fibers is associated with a loss of the pain-producing substance in subcutaneous perfusate collected from damaged areas (Chapman et al., 1961). Furthermore, bradykinin is highly potent for activating peripheral afferent fibers but does not have a selective action on nociceptive afferents because A-beta mechanoreceptive afferents as well as nociceptive fibers also are activated (Hiss and Mense, 1976). Bradykinin applications produce excitatory effects in the majority of TRG neurons in culture (Baccaglini and Hogan, 1983). On the other hand, it was recently shown using autoradiography that receptors for bradykinin are only associated with a subset of small diameter neurons in the TRG of guinea pigs (Steranka et al., 1988).

Tachyphylaxis or refractoriness are not observed in responses to repeated applications of bradykinin, whereas both events are prominent features of responses to applications of ACh, histamine and 5-HT (Keele and Armstrong, 1964; Hiss and Menze, 1976). Therefore, different receptor sites are involved in the activation of nociceptive afferents by these compounds (Hiss and Menze, 1976). The fast adaptation of peripheral nerve fibers to application of the above agents is consistent with the proposal that these algogens may be responsible for the initial fast pain felt after a damaging stimulus. It is likely that the above mentioned substances may all contribute something to the final painful response (Perl, 1976).

1.7.7 Substance P. The observations of Chapman and his colleagues (1961) revealed that the presence of afferent fibers in the vicinity of damaged tissue is a prerequisite for the appearance of a pain-producing substance. This implied a release of the substance from the fibers.
Substance P, a good candidate for the role of the endogenous algogen was already known to be a potent vasodilator and its presence has been established in the peripheral nerve fibers (Pernow, 1953; Gaddum, 1960). This led Lembeck (1953) to propose substance P as the mediator of the vasodilation in the triple response to tissue injury. In addition, substance P was reported to be a potent algogen in studies of the blister base (Armstrong et al., 1954) and in observations of effects following intra-arterial infusions of substance P preparations in mammals (Potter et al., 1962).

Later, it was realised that impure preparations of substance P were contaminated with bradykinin or related kinins (Stewart, 1970). Following the separation and sequencing of substance P (Chang and Leeman, 1970; Chang et al., 1971), the earlier experiments of Armstrong and her colleagues on the human blister base were repeated using synthetic substance P (Stewart et al., 1976). These investigations revealed that substance P did not possess algogenic properties, even at doses up to 1 mg/ml. Substance P application to cat tooth pulp does not produce excitation in the afferent nerve fibers (Gaselius et al., 1977). Thus, the evidence for a role of substance P as the mediator of neurogenic inflammation is very convincing. However, this peptide does not seem likely to be by itself the pain-producing substance in the periphery.

In the present investigations, the actions of several of the above mentioned autacoids were examined for their ability to affect the membrane electrical properties of TRG neurons. These studies were undertaken because the pharmacology of autacoids in sensory ganglia is poorly understood and the mechanisms of actions of such substances on the membranes of primary afferent neurons are virtually unknown.
1.8 Substance P as neurotransmitter of primary afferent neurons

A physiological role for substance P has been of interest since 1931 when the peptide was first isolated by von Euler and Gaddum. There are several reasons for identifying substance P as a neurotransmitter, particularly in sensory systems. Substance P is generated from a larger precursor peptide in small diameter perikarya of sensory ganglionic neurons (Harmar et al., 1981) and transported along their peripheral and central processes (Holton, 1959; Brimijoin et al., 1980). Immunoreactivity for substance P is localized in synaptic vesicles (Cuello et al., 1977), axonal terminations without clearly defined synaptic structures and neuronal contacts with blood vessels (Barber et al., 1979). Peripheral (Olgart et al., 1977), and central (Otsuka and Konishi, 1976) release of substance P has been demonstrated on electrical stimulation of the peripheral nerve fibers. Motoneurons in the spinal cord and neurons of the cuneate nucleus in the brainstem are depolarized on application of substance P (Konishi and Otsuka, 1974; Krnjević, 1977). Furthermore, an association of the excitatory actions of substance P with nociceptive synaptic inputs has been observed in second order sensory neurons of the spinal trigeminal tract (Andersen et al., 1977; 1978). Also, the excitabilities of terminals of primary afferent fibers were shown to be greatly affected by substance P applications (Randić et al., 1982). However, sensory neurons themselves do not respond to substance P applications in in vitro preparations (Krishtal and Pidoplichko, 1981; Nowak and Macdonald, 1982).

A mediator role for substance P is likely at synapses of sensory afferent fibers with neurons of inferior mesenteric ganglia that are depolarized by its application (Dun and Karczmar, 1979; Krier and Szurszewski, 1979; Konishi et al., 1979); the non-cholinergic excitatory postsynaptic
potentials are evidently generated by the postganglionic actions of
substance P released by dorsal root stimulation (Konishi et al., 1980).

1.9 Membrane electrical properties of primary sensory neurons

The membrane electrical properties of DRG neurons were among the first
to be studied with intracellular recording techniques (Svaetichin, 1951).
Several features of DRG neurons have made them readily accessible to electrophysiological analyses. The presence of only a single stem process which is attached to a spherical or ovoid perikaryon facilitates the analysis of electrophysiological membrane properties when intracellular recordings are employed. This relatively simple geometry obviates some of the difficulties in interpretation of the voltage responses to intracellular injections of current that are used to assess membrane electrical properties. Synaptic contacts or inputs are virtually absent and this further simplifies analysis and interpretation of results.

The membrane properties of TRG neurons have not been subjected to the same scrutiny as their DRG counterparts because of the notion that perikaryal membrane properties of TRG neurons are similar to those in the DRG, and also due to the relative anatomical inaccessibility of the TRG in many species (e.g. rabbit). However, several features of the TRG neurons make it likely that their membrane properties may differ from those of the DRG neurons. First, neurons in the TRG derive from two embryologically separate cell groups of the neural crest and the ectodermal placodes, whereas DRG neurons derive exclusively from the neural crest. These differences in the embryological origin could result in functional differences.

In humans, and perhaps in other mammals, trigeminal neuralgia (tic douloureux) is a disorder characterized by lightning-like attacks of pain of
extreme intensity that can be precipitated by light stimuli from the skin, face or gums, in an apparent absence of neurological deficit. Although considered by some investigators to have a central pathogenesis (cf. Selby, 1984), more convincing evidence points to peripheral origins of the neuralgia, with a more likely site of pathology within the TRG itself or in its sensory root (Kerr, 1979). In the glosopharyngeal ganglion, which has embryological origins similar to the TRG, neuralgias occur very infrequently, and almost never in other sensory ganglia such as the DRG (Selby, 1984).

The possibility that impulses can be initiated in perikarya of TRG neurons in pathophysiological conditions is inferred from the similarities of some types of epilepsy and trigeminal neuralgia. The clinical efficacies of baclofen and particularly of the antiepileptic agents carbamazepine and phenytoin in the treatment of trigeminal neuralgia (Fromm and Terrence, 1983; Rall and Schleifer, 1985) suggest that these drugs may have inhibitory actions on putative epileptiform discharges in the TRG. Sensory neurons that have sustained some injury to their peripheral processes also would be expected to discharge spike bursts (cf. Burchiel and Russell, 1985). However, investigations of the chronic effects of epileptogenic agents that have been applied directly to the TRG in cats have not revealed any evidence of abnormal spontaneous activity or physiologically evoked bursts of spikes in TRG extracellular recordings (Burchiel et al., 1978). Nevertheless, many convulsants produce their gross effects on motor and sensory function by actions on synapses, which are absent in the TRG (Lieberman, 1976). Also, extracellular recordings as compared to intracellular techniques do not provide a sensitive reflection of changes in membrane properties. Furthermore, spontaneous discharges in sensory neurons have been recorded under certain conditions (cf. section 1.4).
1.9.1 Control of membrane excitability. The transmission of impulse patterns that travel along the primary afferent fibers is dependent on a strict control of membrane excitability at the sites of impulse generation and propagation. The control of neuronal excitability is largely attributable to currents mediated by potassium channels (Hille, 1984). Most of these currents are activated with membrane depolarization and can be partially resolved using voltage clamp techniques because of differences in their kinetics of activation and inactivation. These currents can be separated by changing the ionic species in the extracellular and intracellular environments as well as with selective $K^+$-channel blockers. The most commonly used $K^+$-channel blockers, tetraethylammonium (TEA), 4-aminopyridine (4-AP) and Cs$^+$, have different affinities for various types of $K^+$-channels (Adams and Galvan, 1986). An example of specificity in pharmacological blockade is the transient outward $K^+$-current ($I_A$) in molluscan neurons (Hagiwara et al., 1961), which has been implicated in the control of repetitive spike discharge (Connor and Stevens, 1971; Neher, 1971). In this case, as well as in neurons of vertebrates (except bullfrog, cf. Adams and Galvan, 1986), $I_A$ is usually very susceptible to blockade with 4-aminopyridine (4-AP) and not very sensitive to similar external applications of another $K^+$-channel blocker, tetraethylammonium (TEA). Other $K^+$-currents with kinetics that are suggestive of $I_A$ have been investigated with patch-clamp techniques in dorsal root and nodose ganglion neurons where this pharmacological characteristic, i.e., blockade by 4-AP, was demonstrated (Kasai et al., 1986; Bosu et al., 1985; Oyama, 1987). In contrast to the patch-clamp results, the putative $I_A$ recorded intracellularly in rat nodose neurons is not much affected by applications of 4-AP (Stansfeld et al., 1986).
Voltage-clamped sensory axons do not exhibit currents with features of $I_A$ (Chiu and Ritchie, 1980; Grafe et al., 1985; Brismar and Schwarz, 1985). However, applications of 4-AP to myelinated axons produce repetitive spike discharges (Kocsis et al., 1987) that are suggestive of blocking actions on currents other than $I_A$. Administrations of TEA do not elicit spike discharge, but enhance 4-AP-initiated repetitive firing and block the afterhyperpolarizations (AHPs) that usually follow the repetitive spike discharges.

In our previous investigations on TRG neurons a decrease in their abilities to discharge spikes repetitively in response to current pulse injections was observed with TEA applications which did not block the AHPs following the bursts (Puil and Spigelman, 1988; Puil et al., 1988), suggesting certain differences from other sensory neurons and axons (cf. above). Therefore, one of the main objectives in the present investigations was to examine in detail the effects of applications of different $K^+$-channel blockers (4-AP, TEA and Cs$^+$) on the membrane voltage responses and outward currents of TRG neurons, in order to understand better the fundamental processes that affect their excitabilities, including repetitive firing. The other objective was to examine the effects of applications of several endogenous substances (autacoids) on the excitabilities of TRG neurons.
2 METHODS

2.1 Animals

2.1.1 Source. Duncan Hartley guinea-pigs (either sex) were obtained from the Animal Care Centre of the University of British Columbia. The Animal Care Centre used standard animal care procedures for the maintenance of laboratory animals. At the Centre, guinea-pigs were weaned after 14 days. These animals were fed on vitamin C-supplemented guinea pig chow and had access to water ad libitum.

2.1.2 Animal feed and housing. Once a week, typically on Mondays, 4-6 guinea-pigs (200-300 g, approximately 28 day old) were received from the animal unit and used for studies in that same week. Once the animals were acquired, they were placed in a wire cage (58 x 35 x 53 cm, in size) in the animal care room of the Department of Pharmacology and Therapeutics. These guinea pigs had free access to food (guinea pig chow) and water. The animal care rooms had controlled temperatures (22-23° C) and humidity (50-55%) with set 12-hourly day and night periods.

2.2 Surgical procedures for removal of the TRG

Anesthesia was induced with 4% halothane administered in a 2-liter chamber. The trachea was cannulated and the animal's skull hair removed with scissors. The animal then was placed in a stereotaxic head holder. A craniotomy was performed while anesthesia was maintained endotracheally with 2% halothane. After midcollicular decerebration with a scalpel blade, the encephalon was aspirated to reveal the underlying posterior fossae. At this stage, the administered halothane concentration was reduced to 0.5%. Heart rate was monitored via electrodes inserted subdermally in the thorax region
throughout the surgical procedure in some experiments. The left trigeminal
root ganglion was carefully isolated from the surrounding connective tissue
and cartilagenous bone with the aid of a dissecting microscope and fine
instruments (e.g. No. 5 jeweler's forceps and miniature scalpel blade). Care was taken not to disturb blood vessels in the vicinity of the ganglion
for two reasons: (1) minimization of the duration of hypoxia was considered
desirable, and (2) profuse bleeding interfered with visualization and
dissection of the ganglion. The central and peripheral stumps of the
ganglion were severed such that the total length of the excised tissue was 1
to 1.5 cm. Usually, the central end was cut as close as possible to its
entry into the brainstem. Following total removal from a posterior fossa,
the ganglion was immersed quickly into cold artificial cerebrospinal fluid
(ACSF) which was oxygenated with a 95/5% gaseous mixture of $O_2/CO_2$. The
remaining connective tissue, arachnoidal and dural (capsular) sheaths sur­
rounding the ganglion, and its central and peripheral root stumps, were
removed from the ganglion with the aid of a dissecting microscope (Leitz).
The ganglion was placed on a Teflon stage of a mechanical tissue chopper
which was covered with ACSF-saturated porous tape and then cut parasagittally
into thin (250-450 $\mu$m) slices; this procedure preserved some of the axon
bundles in the stumps of both central and peripheral branches.
Slices were transferred immediately into a beaker (50 ml) with a nylon
mesh immersed in continuously oxygenated ACSF at room temperature (19-24°C)
until required for recording. The procedures for dissection, slicing etc.
of the second ganglion were identical to those described above and were
carried out within 30 min after preparation of slices of the first ganglion.
2.3  Procedure for obtaining human trigeminal root ganglia at autopsy

Trigeminal root ganglia were obtained 4 hrs postmortem from a male donor (age, 81 years) at the autopsy facilities of the Acute Care Hospital, The University of British Columbia. Following removal of the skullcap and the brain, trigeminal root ganglia were excised from the posterior fossae (procedure carried out by Dr. E. Puil). The ganglia, including surrounding connective tissue, were immersed into cold (4°C) ACSF that was oxygenated with 95% O2, 5% CO2 mixture 15 min prior to removal of the ganglia. The remaining dissection and slicing procedures were similar to those employed for trigeminal root ganglia from guinea pigs and took place in the electrophysiological laboratory.

2.4  Procedure for obtaining human sympathetic ganglia

Sympathetic ganglia were harvested from 6 human donors (ages, 9-54 years) of certain organs for the Pacific Organ Retrieval for Transplantation programme. The brain-dead individuals had no recent history of disease, cardiovascular dysfunction or medication and were artificially ventilated for the surgical procedures. Only one neomort had received, intraoperatively, a drug (dopamine) which was used to maintain good peripheral perfusion. Lumbar sympathectomies were performed (by Dr. E. Puil) within 1-2 min after bilateral nephrectomy, i.e., the first procedure in the operating room protocol for organ retrieval. An excised chain of 4 ganglia was immersed in cold (~4°C) oxygenated (95% O2, 5% CO2) artificial cerebrospinal fluid
Fig. 1. Schematic diagram of the transport chamber. The plexiglass chamber was fastened to a gas tank (Linde, size E) containing the gas mixture which was used to oxygenate the artificial cerebrospinal fluid (ACSF). The two layers of nylon mesh were separated by a space of ~1.5 mm.
(ACSF). In an adjacent laboratory, each ganglion with the nerve stumps (~1 cm total) was dissected free of the connective sheaths with the aid of a microscope and sliced longitudinally with a manual chopper, within ~5 min after the sympathectomy. The slices (300-500 μm thick) were transported in a specially designed chamber (Fig. 1) with oxygenated ACSF to the electrophysiological laboratory where they were maintained with constant oxygenation until needed for recording. In one case, the intact ganglia, as above, were stored overnight (~8 hr) at 4°C before slice preparation.

2.5 Electrophysiological recordings

All electrophysiological studies of neurons in the different preparations were performed in a recording chamber (Fig. 2). This chamber (1 ml volume) had a single inlet for inflow of solutions. The outflow from the chamber was by capillary action through a strip of filter paper into an adjacent chamber, where it was removed by aspiration. A nylon mesh prevented floating movement of slices in the chamber during perfusion with artificial cerebrospinal fluid (ACSF) at rates of 1-3 ml/min. Preparations were visualized with a compound microscope (Leitz) equipped with Hoffman modulation contrast optics. The recording assembly was housed in a wire Faraday cage (to prevent 60 Hz electrical interference) on a vibration-damping table (Micro G).

The control ACSF had the following chemical composition (in mM): NaCl, 124; NaHCO₃, 26; KCl, 5.0; KH₂PO₄, 1.25; CaCl₂, 2.0; MgSO₄, 2.0; and dextrose, 10. Continuous bubbling with a 95/5% O₂/CO₂ mixture ensured adequate oxygenation and a pH of 7.4. Bath fluid temperature was
Fig. 2. Schematic representation of the recording chamber. A heating device was installed underneath the glass bottom of the chamber. Short (~15 cm) sections of sylastic tubing connected the perfusion barrels to the polyethylene perfusion lines (inside diameter, 0.86 mm). These lines terminated in a single outlet ~8 cm from the recording chamber. Vascular clamps were used to switch between perfusates from different barrels.
maintained at 32-34°C with a heating device fitted underneath the floor of the recording chamber and by additional heating of the perfusion lines (Fig. 2).

2.5.1 Ion substitutions. Low-Na⁺ solutions were made by equimolar substitution of NaCl with choline chloride. Changes in [K⁺] of the ACSF were made by equimolar substitution of NaCl with KCl. CaCl₂ was replaced with CoCl₂ in low-Ca²⁺ solutions. MgCl₂ was omitted from Mg²⁺-deficient solutions. In several experiments, another 2 mM of CaCl₂ were added to Mg²⁺-deficient solutions.

2.5.2 Drugs. All drugs were obtained from Sigma. Aliquots of substance P and bradykinin were measured from stock solutions (0.1 mM) that were kept frozen until required. The other solutions were prepared on the day of the experiment, or the night before, from drugs that were stored according to drug company recommendations.

2.5.3 Electrodes. The recording electrodes were pulled on a microelectrode puller (Narishige PA-81) from borosilicate glass pipettes (WPI or Frederick Haer, 1-1.2 mm outside diameter). These electrodes were filled with 3 M KCl, 3 M CsCl, 4 M K₂SO₄ or 3 M Cs₂SO₄, and had tip resistances ranging from 12 to 35 MΩ. Voltage-clamp experiments were performed using electrodes with tip resistances no greater than 25 MΩ, whereas electrodes with higher tip resistances were used in current-clamp experiments. Penetration of neurons was achieved with the aid of a micropositioner (Burleigh Instruments). Brief bursts of capacitance overcompensation were used to facilitate penetration. A chlorided silver wire served as the ground electrode. In some experiments, concentric bipolar stimulating electrodes (Rhodes Medical Instruments) were inserted into the peripheral or central stumps of TRG slices. Applied voltages of 0.5-6 V were used to
elicit action potentials recorded in the cell body.

2.5.4 Recording equipment. In several early experiments, the microprobe amplifier system (WP Instruments M701) which was used to measure potentials, allowed injection of current via a bridge-balance circuit for conventional measurement of membrane resistance with constant current pulses. In subsequent experiments, specified voltage or current steps were injected through the recording electrode with the Axoclamp-2A amplifier (Axon Instruments) in either the current-clamp or single electrode voltage-clamp (sampling frequency 2.5-3.5 kHz) mode. Voltage and current recordings were monitored continuously with a chart recorder (Gould Brush 220 or Hewlett-Packard 7404A). Amplified potentials were stored on FM tape (Sony or Scotch) using a tape recorder (Hewlett-Packard 3968A) that had a frequency response flat to 5 KHz at 7.5 ips. For detailed data analysis, intracellular recordings were played back via a waveform recorder (Biomation 805) and reproduced on paper with an X-Y pen recorder (Hewlett-Packard 7015B). Alternatively, signals were averaged by computer (MINK-23; PDP 11/44).
3 RESULTS

3.1 Dependence of resting membrane potential on extracellular cations.

Data were obtained from TRG neurons that showed stable resting potentials more negative than -50 mV and spike amplitudes greater than 50 mV for periods of 5-180 min. In all cases, the extracellular voltage level was determined following withdrawal of an electrode from the cell. Recordings were performed using KCl-filled electrodes, unless otherwise indicated. The input resistance of neurons was estimated from the voltage responses to injections of small (0.1-0.5 nA) hyperpolarizing current pulses (50 ms duration).

3.1.1 Changes in extracellular [K⁺]. The effects of changes in the extracellular concentration of K⁺ were studied in 10 neurons. In 8 neurons, the extracellular [K⁺] was varied by changing to perfusates containing 20, 30, or 40 mM K⁺. In each case, a steady level of membrane potential was attained at 3 min of perfusion. The mean values of resting potential for these 8 neurons are plotted against the concentration of K⁺ in the perfusate (Fig. 3). In separate experiments on two other neurons, complete removal of K⁺ from the perfusate resulted in a hyperpolarization to -79 mV and -104 mV. The depolarizations in high [K⁺] solutions (e.g. 20 mM) were accompanied by decreases in input resistance. In 8 neurons the mean input resistance was decreased from 12 ±2.4 to 5.9 ±0.7 MΩ S. E. M. It is predicted from the constant field equation that for 5 mV hyperpolarizing test pulses the K⁺-conductance ratio increases by a factor of 1.69. If the Cl⁻ is passively distributed, the Cl⁻-conductance ratio would be 1.47. In 6 of 7 cells the conductance ratios for 20 mM [K⁺] and 6.25 mM [K⁺] were greater than the conductance ratios expected from the
Fig. 3. Dependence of membrane potential on extracellular [K⁺]. Each point on the graph represents the mean and S. E. M. of membrane potential measurements for 8 TRG neurons obtained at different concentrations of K⁺ in the perfusate. The extrapolated best line fit to the points in the graph intersects the ordinate at -77 mV.
constant field equation (assuming 100% membrane potential dependence on $K^+$ and unaltered $K^+$-permeability). However, the differences were not significant (student t-test). Therefore, these data do not support an increased $K^+$-permeability (i.e., no opening of channels with depolarization in this range).

3.1.2 Changes in extracellular $[Na^+]$. Perfusion of neurons with solutions where choline chloride was substituted for NaCl resulted in a 2 mV hyperpolarization of the resting potential in 8/10 neurons. These hyperpolarizations could be recorded in the presence of TTX (1 µM, 5 min), suggesting that the effects were not due to the $Na^+$-channels responsible for action potential generation. In most neurons a slow membrane depolarization ensued ~10 min following the switch to a low-$Na^+$ perfusate. Return to control solution usually restored the membrane potential to its control values. Increases in input resistance were observed on perfusion with low-$[Na^+]$ solutions in 5 neurons (8.8 ±2.2 to 10.1 ±2.5 MΩ S. E. M.) In all cells tested with supra-threshold depolarizing current pulses, the evoked action potentials were abolished by low-$Na^+$ perfusion, although a local membrane response remained (Fig. 4).

3.1.3 Changes in extracellular $[Ca^{2+}]$ and $Co^{2+}$-application. Perfusion of low-$[Ca^{2+}]$, $Co^{2+}$-containing solutions had a hyperpolarizing influence on the membrane potential of TRG neurons. The amplitude of the hyperpolarization was only slightly greater than that observed in $Na^+$-deficient media (2.6 ±0.5 mV S. E. M., n = 7). Also, the hyperpolarization frequently declined despite continued perfusion and reverted into a depolarization that could be halted by returning to control perfusate.
Fig. 4. Effects of low-[Na\(^+\)] perfusate on the voltage responses of a TRG neuron. Perfusion with solution containing low-[Na\(^+\)] resulted in suppression of the evoked spike. The resting membrane potential was -53 mV in control and -55 mV in the low-[Na\(^+\)] perfusates, respectively. Note the slight reduction of the voltage sag in response to hyperpolarizing current pulses in Na\(^+\)-deficient perfusate.
3.2 Membrane properties of TRG neurons

All TRG neurons exhibited varying degrees of time-dependent membrane rectification; this was evident from their 'sagging' voltage responses to hyperpolarizing current pulse injections. Examples are shown in Figs. 4 and 22. Due to presence of a sag at large hyperpolarizations, estimates of input resistance were obtained from voltage responses where sag behavior was not prominent, i.e. responses of <10 mV amplitude. The average input resistance was 8.9 ±0.5 MΩ S. E. M. (n = 83). Many neurons with high values of input resistance were impaled (e.g. 20-35 MΩ), but stable recordings could not be maintained for long periods in such cases, presumably because of cell damage by the relatively coarse electrode tips. Therefore electrode sampling bias probably resulted in an underestimate of the number of cells with high values of input resistance.

3.2.1 Membrane potential oscillations. A tendency for the membrane potential to oscillate was observed when neurons were depolarized by intracellular current injections (Figs. 7 and 8). The oscillations were evident in both the sub- and suprathreshold responses of most neurons, and were prominent in neurons that could discharge repetitively in response to depolarizing current pulses.

3.2.2 Action potentials and afterhyperpolarizations. Two types of action potentials could be distinguished by an absence or presence of a hump on the repolarization phase of a spike evoked by an intracellular depolarizing current pulse (Fig. 5). The majority (>85%) of neurons exhibited spikes with a continuously smooth repolarizing phase (Figs. 5A-C). The slowing of repolarization in humped spikes (Fig. 5D) was very similar to that observed in TRG neurons after interference with K⁺-conductances (see below).
Fig. 5. A and D: two types of action potentials observed in TRG neurons. Arrows indicate onset of intracellularly injected depolarizing current pulses. Note the long spike duration and the presence of a hump during late repolarization just below 0 mV (dashed line) of spike in D. B and C: examples of a single spike and repetitive firing in another neuron exhibiting fast spikes as in A. E and F: examples of a single spike and repetitive firing in another neuron exhibiting humped spikes as in D.
Some characteristics for these spikes are summarized in Table I. The maximum amplitude and duration of spikes including the AHPs were significantly greater in neurons where the repolarization of the spike was biphasic. Mean resting potentials did not differ significantly for the two groups of neurons.

Spike discharge at high frequencies (100-240/s) was elicited from neurons that exhibited either humped or non-humped spikes. However, the apparent threshold for spike generation was higher in cells with non-humped spikes; in addition, the maximal rate of spike discharge was greater than in cells possessing humped spikes (cf. Fig. 5C and F). Long-lasting post-spike AHPs, comparable in peak magnitude and duration to those following repetitive spike discharge in other sensory ganglia (Jaffe and Sampson, 1976; Weinreich, 1983), were not prominent in the TRG neurons. However, AHPs (3-10 mV peak amplitude; cf. Fig. 5C and F) often followed a repetitive discharge evoked by injections (e.g., 1/s for 30 s) of intracellular depolarizing current pulses. Such AHPs lasted about 100-150 ms on termination of a current pulse.

3.2.3 Effects of ionic channel blockade on spikes. TRG neurons exhibited a differential sensitivity to tetrodotoxin (TTX, 1 μM). In 24 neurons, complete blockade of action potentials was observed within 1 min after starting TTX-perfusion. A return of a neuron's ability to discharge spikes of control amplitude was slow, and the amount of depolarizing current needed to evoke a spike remained elevated for more than 20 min after discontinuing the TTX application. The spikes in 15 other neurons were not blocked by similar applications of TTX, even after more than 10-20 min of perfusion. Both TTX-sensitive and TTX insensitive spikes were blocked after 5 min of
<table>
<thead>
<tr>
<th></th>
<th>resting membrane potential (mV)</th>
<th>Action potential</th>
<th>AHP</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>amplitude</td>
<td>overshoot</td>
<td>duration$^1$</td>
<td>amplitude</td>
<td>duration</td>
</tr>
<tr>
<td></td>
<td>(mV)</td>
<td>(mV)</td>
<td>(ms)</td>
<td>(mV)</td>
<td>(ms)</td>
</tr>
<tr>
<td>Fast spikes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>-50 to -75</td>
<td>55 to 104</td>
<td>4 - 45</td>
<td>0.3 - 1.3</td>
<td>1 - 18</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>-60.0 ± 0.5</td>
<td>78.7 ± 1.0</td>
<td>17.6 ± 1.0</td>
<td>0.6 ± 0.1</td>
<td>11.9 ± 0.4</td>
</tr>
<tr>
<td>Number</td>
<td>106</td>
<td>118</td>
<td>106</td>
<td>113</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>of cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humped spikes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>-53 to -70</td>
<td>70 to 101</td>
<td>8 - 43</td>
<td>0.9 - 4</td>
<td>5 - 25</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>-59.4 ± 1.7**</td>
<td>82.7 ± 1.9*</td>
<td>23.9 ± 2.3*</td>
<td>1.8 ± 0.2*</td>
<td>13.9 ± 1.6*</td>
</tr>
<tr>
<td>Number</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>of cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ at one-half peak spike amplitude.

* $p < 0.05$ (significant difference between means of fast and humped spike parameters were determined with Student's t-test).

** $p > 0.05$. 

perfusion with solutions containing low-[Na⁺] (Fig. 4). The spikes were unaffected during presumed Ca²⁺-channel blockade with low-[Ca²⁺], Co²⁺- containing perfusates (n = 7). However, a slight deterioration of spike amplitude was observed after prolonged periods (>5 min) of perfusion with the low-[Ca²⁺] solutions.

3.3 Membrane properties of human sympathetic neurons.

Stable recordings were obtained from 16 neurons that had resting membrane potentials in the range of -28 to -65 mV (mean, -45 ± 2.8 mV S. E. M.), for stable periods of at least 10 min to ~12 hr. Several resting potentials of ~-10 to -20 mV were recorded in the slices of the ganglia that had been stored overnight at 4°C, but spontaneous activity was not observed and spikes could not be evoked by current injections. Spontaneous activity also was not observed in most neurons of the other "acute" preparations, although positive transients of ~5-6 mV amplitude were suggestive of synaptic activity in 1 neuron.

The neurons exhibited average input resistance of 28.7 ± 4.6 MΩ (S. E. M.) when injected with hyperpolarizing current pulses. The responses to these pulses generally were 8-10 mV in amplitude and usually followed an approximately exponential time course (Figs. 6A, 15A and C). A membrane time constant of 13.2 ± 1.0 ms (S. E. M.) was calculated for 3 neurons by measuring the time taken to reach 68.8% of peak voltage responses (<20 mV) to hyperpolarizing current pulses. Hyperpolarizing responses of >20 mV amplitude sometimes sagged, indicative of time-dependent activation of a voltage-sensitive conductance (Fig. 6C).

Anodal break responses often consisted of a spike of >60 mV amplitude on the depolarizing rebound, followed by a slow, pronounced hyperpolarization.
FIG. 6. Responses of human neurons to injections of square wave current pulses. A-C: anodal break responses, including spikes displaying prominent afterhyperpolarizations in 3 human sympathetic neurons (same neuron in C and D). Hyperpolarizing current pulses (100 ms) were 0.2, 0.3 and 1.7 nA in A, B and C, respectively. D: depolarizing current injections (100 ms) were 0.3, 0.8 and 1.3 nA for D1, D2 and D3, respectively. Resting membrane potential was -65 mV. Upper dashed lines represent 0 mV level.
With graded injections of current steps, a progressive delay in the appearance of the spike could be demonstrated (Fig. 15A and C). Such spikes and afterhyperpolarizations (AHPs) could be more easily activated with the smaller test pulses. In some neurons, the anodal spike disappeared entirely with large current pulse injections (Fig. 14C), unlike the anodal spikes in TRG neurons which were more easily evoked by large test pulses. The AHPs had large amplitudes (e.g., >20 mV in Fig. 6A) and long durations (50-400 ms) even in neurons with resting potentials of ~-50 to 65 mV.

In general, and compared with the spikes that were part of the anodal break responses, spikes were less readily evoked with intracellular injections of depolarizing current pulses. Such pulses generated spikes of >40 mV amplitude in many of these neurons (Fig. 6D). The sensitivities of the spikes to Na\(^+\)-channel blockade with TTX application were observed in 2 cells. The anodal break and the directly evoked spikes were blocked after 2.5 minutes of perfusion with TTX (1 μM). Recovery from TTX blockade was observed 8 minutes after return to control solution.

In some neurons, repetitive firing could be generated with large current pulses such as in the case of Fig. 6D. Termination of the depolarizing current injections was followed by slow hyperpolarizations. The amplitude and duration of these "AHPs" increased with the number of spikes in the train (Fig. 6D1-D3).

3.4 Effects of K\(^+\)-channel blockers

3.4.1 Current clamp: effects of tetraethylammonium on subthreshold potentials. In contrast to the effects of TTX in TRG neurons, administration of TEA (5-10 mM) blocked the subthreshold oscillations on injection of depolarizing current pulses (Figs. 7D and 8A) and did not
Fig. 7. Effects of combined application of TTX (1 μM) and TEA (10 mM) on oscillatory membrane potential responses and spikes evoked by intracellular depolarizing current pulses. Spikes of this TRG neuron were unaltered by TTX applied for 20 min. A-D: superimposed oscilloscope traces show membrane responses to sub- and suprathreshold stimuli. Note that membrane potential oscillations persist in TTX perfusate and are blocked by TEA application. E: suprathreshold response shows reduction in repetitive firing ability compared with C. F: partial recovery from the effects of TEA was evident 5 min after return to control perfusate as an increase in evoked repetitive firing and a reduction in spike duration compared with E. Initial resting membrane potential was -52 mV. The amplitudes of suprathreshold current pulses were approximately 2.0 nA in A and D, 2.2 nA in B, C and E, and 2.1 nA in F.
### Table II. Effects of tetraethylammonium (TEA; 10 mM) on electrical properties

<table>
<thead>
<tr>
<th></th>
<th>resting membrane potential (mV)</th>
<th>input resistance (M)</th>
<th>Action potential amplitude (mV)</th>
<th>overshoot (mV)</th>
<th>duration (ms)</th>
<th>AHP amplitude (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>mean ± S.E. -57.4 ± 1.8</td>
<td>12.1 ± 1.5</td>
<td>74 ± 2.8</td>
<td>16 ± 3.4</td>
<td>1.1 ± 0.3</td>
<td>11 ± 2.0</td>
</tr>
<tr>
<td><strong>TEA</strong></td>
<td>mean ± S.E. -54.7 ± 1.8</td>
<td>16.9 ± 1.9</td>
<td>78 ± 3.6</td>
<td>20 ± 3.6</td>
<td>2.4 ± 0.6</td>
<td>3 ± 1.0</td>
</tr>
<tr>
<td><strong>number of cells</strong></td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>
affect the sag in the hyperpolarizing responses to current pulses. TEA (10 mM) application resulted in 47% increase in the mean input resistance of 10 neurons (Table II). Typically this increase was associated with a 2-5 mV depolarization.

3.4.2 Effects of TEA on spikes and repetitive discharge. The effects of external TEA (10 mM) application were assessed on the spike characteristics of 10 neurons. The rising phase of evoked spikes was not affected. An increase in the spike overshoot was evident after TEA application, and was attributable, at least in part, to an increased input resistance. A prolongation of the falling phase of spikes and a reduction in peak amplitude of the spike AHPs were observed in all cases. The prolonged mean spike duration and the reduction in the mean amplitude of the AHPs measured at one-half peak amplitude were 1.3 ms and 8 mV, respectively, after 3-5 min of perfusion with TEA (10 mM).

During TEA administration, enhancement of repetitive spike discharge was not observed during suprathreshold current pulse injections despite the induced depolarization (2-5 mV), reduced spike threshold, and increased input resistance. In fact, TEA application produced a decrease in the ability of neurons to discharge repetitively (Fig. 7). Recovery from a TEA application was rapid and usually complete after 5-6 min.

The effects of TEA (10 mM) on spikes evoked in human sympathetic ganglion neurons by current injection were studied in 4 neurons. A slight increase in input resistance (6.6%; range, 0-17%) and an increased spike amplitude (cf. Fig. 9) was observed during TEA (10 mM) perfusion. Spikes were broadened, sometimes approaching 10 ms in duration and the repolarization phase was composed of two distinct slopes during TEA perfusion (Fig. 9).
FIG. 8. Comparison of TEA and 4-AP effects on spikes and subthreshold oscillations. A: responses to sub- and suprathreshold injections of current pulses in the presence and absence of TEA (5 mM) and 4-AP (5 mM). During drug application, the membrane potential was hyperpolarized with direct current (DC) injection to control levels. TEA application (at 2 min) abolished the subthreshold oscillations, reduced spike threshold, increased spike duration and reduced AHP amplitude. Following recovery from the effects of TEA (not shown), application of 4-AP (at 2 min) produced a large increase in the subthreshold oscillations, a decrease in spike threshold and a slight increase in spike duration, but did not reduce spike AHPs. B: responses of another neuron to application of 4-AP (1 mM) in the absence of DC injection. The resting potential was depolarized by several millivolts and a large increase in repetitive spike discharge was observed.
Fig. 9. Effects of TEA (10 mM for 3 min) and 4-AP (1 mM for 6 min) on directly evoked spikes from a human sympathetic neuron. Current pulses were 0.9 nA in control, 0.7 nA in TEA and 4-AP perfusates. Membrane potential was maintained at -65 mV using DC injection. Input resistance was 32 MΩ, 39 MΩ and 43 MΩ in control, TEA and 4-AP perfusates, respectively.
Also, TEA applications produced a slight reduction in the amplitude of the spike AHPs.

3.4.3 Effects of 4-aminopyridine on subthreshold potentials of TRG neurons. Applications of 4-AP (1 mM) usually depolarized neurons by 3-9 mV (4.9 ±0.9 mV S. E. M., n = 6). The input resistance was increased from 7 ±0.3 to 11 ±0.6 MΩ S. E. M. (n = 4). The subthreshold potential oscillations resulting from intracellular injections of depolarizing current pulses were greatly increased (Fig. 8A, B), in contrast to the effects of TEA. These increased oscillations were not a result of membrane depolarization as they were observed even when the membrane potential was compensated with hyperpolarizing direct current injection (Fig. 8A).

3.4.4 Effects of 4-AP on spikes and repetitive discharge. Injections of suprathreshold depolarizing current pulses into many neurons often elicited multiple spike firing (cf. Figs. 7, 8, 10) that was facilitated by an application of 4-AP. In other neurons, such injections evoked only a single spike; however, in the presence of 4-AP, these neurons also exhibited repetitive spike firing as shown in Fig. 8B. Spikes tended to sit on top of the oscillations. Spike duration was slightly increased but the AHPs were not reduced. Furthermore, applications of 4-AP (1-5 mM) elicited spontaneous spike activity in 4 neurons (cf. Figs. 10, 24). The enhancement of repetitive discharge ability and the lack of major effects on the AHPs were in direct contrast to the effects of TEA applications. Some recovery was observed in most cases at 5-30 min after termination of a 4-AP application. Full recovery sometimes was observed at 30-40 min after a low dose application (<0.1 mM) in several neurons.
Fig. 10. K⁺-channel blockade results in repetitive spike discharge and burst activity in a TRG neuron. A: In control conditions, discharges elicited with intracellular injections of depolarizing current pulses of 3 different amplitudes show phenomenon similar to spike accommodation. Note oscillation at the termination of spike activity. A maximal discharge rate of ~240 Hz was obtained with the pulse illustrated at the righthand side. B, left: continuous spiking was evident at ~3.5 min after starting 4-AP perfusion (1 mM). B, right: repetitive activity was replaced by bursts at ~10s intervals on hyperpolarizing the neuron with direct current (DC) injection. Note long interspike interval before termination of the burst. C, left: application of TEA (10 mM) concomitantly with 4-AP led to irregular spikes in bursts at ~5-20s intervals and long afterhyperpolarizations. Injection of DC to potentials more negative than rest (e.g., <-75 mV) did not arrest bursts, but prolonged "spontaneous" depolarizing potentials and afterhyperpolarizations.
Application of 4-AP to human sympathetic neurons also slightly increased the duration of the spikes, and unlike TEA applications, did not greatly affect their shapes or AHPs (cf. Fig. 9). With washout, the effects of 4-AP persisted for more than 20 min, whereas the recovery from the effects of TEA usually was complete within 5-8 min.

3.4.5 Combined applications of 4-AP and TEA. One of the prominent features of a concomitant application of 4-AP (1-5 mM) and TEA (5-10 mM) was the increase in the ability of neurons (n = 5) to discharge slow spikes, presumably mediated by Ca\(^{2+}\), in response to current pulse injections. These spikes were evoked in cases where prior applications of TTX (1 μM) blocked the evoked spikes (Fig. 11). An increase in the duration of these Ca\(^{2+}\)-spikes also was observed in cells that discharged spontaneously following application of 4-AP. As shown for the neuron in Fig. 10C, the combined application of 4-AP (5 mM) and TEA (5 mM) transformed the continuous spike activity into a burst-like pattern of spikes followed by a long hyperpolarization at regular interburst intervals of 5-20 s. When this neuron was hyperpolarized by ~20 mV using DC injection, the bursts of spikes induced by the combined application of 4-AP and TEA were replaced with initial few spikes and oscillations that rode the crest of a single, prolonged depolarization similar to cardiac action potentials. Also, these slow depolarizations were followed by long hyperpolarizations resembling AHPs. In the cell of Fig. 10, the spontaneous depolarizations including spikes and bursts were completely suppressed by a 3 min application of TTX (1 μM).
Fig. 11. Effects of Ca\textsuperscript{2+}-channel blockade on the spikes evoked during the combined application of 4-AP (5 mM), TEA (10 mM) and TTX (1 μM). The membrane potential was maintained with DC injection at -55 mV, -53 mV and -52 mV in control, 0 [Ca\textsuperscript{2+}], Co\textsuperscript{2+}-containing and recovery perfusates, respectively. Note that the hyperpolarization that follows the large depolarization in the absence of a Ca\textsuperscript{2+}-spike is of a smaller amplitude than that of AHPs following spikes.
3.4.6 Effects of intracellular Cs$^+$. Intracellular injections of Cs$^+$ (1-4 nA, 50-100 ms depolarizing pulses delivered at 1/s) or application by leakage from the recording pipette resulted in a progressive increase in input resistance, depolarization, as well as a reduction in the amplitude of spikes. Neurons studied with Cs$^+$-containing electrodes were depolarized by 22 to 35 mV within 8 min after the initial impalement ($n = 12$). Nevertheless, when the membrane potential was compensated with hyperpolarizing direct current injection, the amplitude of spikes was greater compared to the amplitude of spikes evoked at the early stages of recording. The duration of evoked spikes was prolonged and the amplitude of AHPs following spikes was reduced by Cs$^+$-application (Fig. 12). As with TEA administration, an increase in spike overshoot was observed with Cs$^+$ application, probably a consequence of the increased input resistance. An effect common to both types of K$^+$-channel blockade was a transformation of non-humped spikes to humped spikes. The initial part (10-20 mV of repolarization (cf. Figs. 7, 12) was more "resistant" than the rest of the falling phase (i.e., toward the resting potential) to the actions of TEA or Cs$^+$.

In contrast to the effects of combined applications of 4-AP and TEA, Cs$^+$ applications resulted in the progressive blockade of the prolonged spike afterhyperpolarizations. Indeed, spikes recorded a short time after blockade of the afterhyperpolarizations with CsCl or Cs$_2$SO$_4$-filled electrodes were followed by prolonged afterdepolarizations (Fig. 13B and C). Perfusion with low-[Ca$^{2+}$], Co$^{2+}$-containing solutions resulted in blockade of the spikes including the afterdepolarizations.
Fig. 12. Action potentials evoked in a TRG neuron at different times after initial impalement of a neuron with a 3 M CsCl electrode. Top traces represent differentiated traces of lower records. Blockade of the AHPs as well as spike prolongation was evident after 9 min. Note that the repolarization phase in the second action potential is greatly prolonged relative to that of the spike evoked at the onset of depolarizing current pulse. Dashed lines represent 0 mV potential.
Fig. 13. Spike afterpotentials in TRG neurons.

A: depolarizing current pulse evoked a $\text{Ca}^{2+}$-spike in the presence of 4-AP (5 mM), TEA (5 mM) and TTX (1 $\mu$M). Such spikes were blocked in a low-[Ca$^{2+}$], Co$^{2+}$-containing medium. Note the long lasting afterhyperpolarization that follows the termination of the current pulse. B: in another neuron, a $\text{Ca}^{2+}$-spike evoked by a depolarizing current pulse injected through a CsCl-containing electrode is followed by a prolonged afterdepolarization. C: depolarizing current pulse injected through a Cs$\text{SO}_4$-containing microelectrode also produces an afterdepolarization. The membrane resting potential was held at -50 mV, -60 mV and -62 mV in A, B and C, respectively.
A

4-AP
TEA
TTX

KCl electrode

---

B

4-AP
TEA
TTX
low-Na^+

CsCl electrode

---

C

100 ms

30 mV

Cs_2SO_4 electrode
3.5 **Voltage-clamp analysis**

TRG neurons \((n = 35)\) were investigated with single electrode voltage-clamp techniques during perfusion with TTX (1 μM) in order to identify the specific currents affected by the \(K^{+}\)-channel blockers. These experiments required a trade-off between the current passing abilities of the recording electrodes and minimization of damage to impaled neurons. However, the low input resistances of TRG neurons resulted in further complications (cf. neurons in the CNS or sympathetic ganglia). For these reasons, the voltage-clamp was imperfect; an effective space-clamp could not always be achieved despite the approximately spherical perikarya, and further resulted in a sampling bias that yielded rather low numbers of successfully clamped neurons. The use of \(K^{+}\)-channel blockers resulted in an improvement of the clamp characteristics due to the increase in neuronal input resistance.

3.5.1 **Transient outward currents**. One outward current could be isolated from the others by simple voltage conditioning. For example, when the membrane potential was held at about -40 mV, hyperpolarizing voltage commands evoked inward currents that were followed by outward currents at the termination of the commands (Fig. 14A). These transient currents had a rapid onset (<5 ms) and were inactivated within ~50 ms. The mean decay time constant of the transient outward currents estimated for 5 neurons was 19.3 ±6.3 ms S. E. M. These estimates were obtained from 4-7 averages of current traces that were fitted with a single exponential (~0.9 correlation).

The presence of inward (anomalous) rectification did not allow the use of hyperpolarizing voltage commands greater than 35 mV because activation of an inward rectifier current interfered with the transient outward currents (cf. Fig. 14A, bottom), and was the likely cause for a slight deviation in the transient current decay from a single exponential.
A reduction in the inactivation of the transient outward currents could be achieved with hyperpolarizing commands as small as 5 mV from holding potentials near -40 mV. The time constant for the removal of inactivation was determined by varying the duration of hyperpolarizing voltage commands of constant amplitude (Fig. 14B). The use of this paradigm yielded an average decay time constant value of 29 ± 7 ms in 3 neurons. Due to the limitations of the single electrode voltage clamp, no attempt was made to investigate steady-state activation or inactivation of the transient outward currents.

Human sympathetic neurons also exhibited transient outward currents. In two neurons that could be successfully voltage-clamped, termination of the larger hyperpolarizing voltage commands from a holding potential of -40 mV was accompanied by transient outward currents of 1-3 nA amplitude (Fig. 15B). The durations of the small transient currents were briefer (eg. <40 ms) than the latencies of the anodal spike responses to the injected pulses using bridge-balance techniques.

3.5.2 Effects of ionic substitution. When [K⁺] in the perfusate was increased to 20 mM, a reduction was observed in the transient outward currents of TRG neurons. The peak amplitudes and decay of the currents were only slightly (<25%) reduced by perfusion with solutions in which 2 mM CoCl₂ had been used as a substitute for CaCl₂ (n = 2). A small outward shift in steady-state holding current usually was observed on perfusion with the low-[Ca²⁺], Co²⁺-containing solutions. The holding current also was shifted in an outward direction during perfusion with low-[Na⁺] media. These shifts corresponded to the small membrane hyperpolarizations that were observed in ionic substitution experiments using bridge-balance techniques.
Fig. 14. Transient outward currents in TRG neurons.

A: hyperpolarizing voltage commands (upper traces) and the corresponding currents (lower traces) evoked from a holding potential of -40 mV. A transient outward current was evoked at the termination of the commands. The decay of this current was approximately exponential and nearly complete by ~50 ms. Large hyperpolarizing commands activated a slow inward current that contributed to a small reduction of the tail current. The graph (below) represents the peak amplitude of the transient current plotted against the amplitude of the command step. B: changes in the duration of constant amplitude hyperpolarizing voltage command steps produced changes in the peak amplitude of the outward current. The peak outward current amplitude is plotted against the duration of the voltage command step in the lower graph. The time constant for the removal of inactivation was ~35 ms (obtained from the best line-fit to the points in the graph).
Fig. 15. Transient outward current in human sympathetic ganglion neurons. A: responses of a human sympathetic neuron to hyperpolarizing current pulse injections. Note the approximately exponential time course at onset of voltage responses, and not on termination of the current pulses. Spike appearance was delayed by pulses of larger amplitude (cf. A and C). B: single electrode voltage clamp recording in another neuron revealed outward currents (lower record) following the two largest voltage command steps (upper record). Holding potential, -40 mV. C: voltage traces selected from A illustrate the progressive delay of spike generation with larger hyperpolarizations. The calibration in A also applies to traces in B and C.
3.5.3 Ineffectiveness of muscarinic agents. Applications of acetylcholine (100 μM, n = 2) and muscarine (20 μM, n = 1) had no effect on the transient outward current. In experiments on other neurons using current-clamp techniques, applications of acetylcholine (100 μM, n = 3), muscarine (50 μM, n = 2) and methacholine (20 μM, n = 1) did not produce changes in the resting potentials or input resistance as assessed from constant current pulse injections. Likewise, no changes in the spike characteristics were observed following application of these compounds.

3.5.4 Effects of 4-AP and TEA. Applications of 4-AP had only minor and mostly unspecific effects on the transient outward current; at a relatively high concentration of 5 mM (n = 3), the transient current was unaffected and a decrease in the steady-state current was observed (Fig. 16A, B). In contrast, TEA applications (5 mM) produced a much greater blockade of the transient outward current (n = 3), and elicited a smaller inward shift in the steady-state current (Fig 16C, D).

The possibility that the effects would be dose-specific was assessed from the dose-response relationships for both drugs (Fig. 17). The percentage of inhibition of the transient outward current was determined in the presence and absence of the K⁺-channel blockers, from the slope conductance in these relationships. The dose-response curves in Fig. 17 show that TEA was more potent than 4-AP as a blocker of the transient outward current. In this comparison, the blockade of the current by 4-AP application was less specific because the maximum inhibition of slope conductance that could be obtained was less than 40%. No inhibition was observed on application of 4-AP in 5 mM doses. This decrease in blocking effectiveness may be attributable to membrane breakdown phenomena observed at higher doses of 4-AP (cf. Puil et al., 1988).
Fig. 16. A: single electrode voltage clamp records during control and 4-AP (5 mM) application. Holding potential was -40 mV. Top traces are voltage commands and bottom traces, evoked currents. Transient outward currents were evoked at the termination of the command steps. 4-AP application produced a large decrease in the steady-state and evoked inward currents but had little effect on the transient currents. B: plot of the peak outward current amplitudes against the amplitudes of the voltage commands. Open circles joined by solid lines are control responses and solid triangles with dashed lines, responses obtained during 4-AP application. C: blockade of tail currents by TEA application. Note that TEA produced only a small decrease in the steady-state current. D: graph shows the large TEA-induced reduction in the peak outward currents (solid circles, dashed lines) compared to control (open circles, solid lines).
Fig. 17. The reduction of the peak amplitude of the transient outward current by TEA (open circles) and 4-AP (closed circles). Inhibition of the slope conductance was obtained from I/V relationships in the absence and after 2-3 min of perfusion with a K⁺-channel blocker. Individual points represent the mean of several cells. The number of cells tested is indicated near the points. For several points the standard errors were very small. Curves are computer generated lines (2nd order regression) fitted to the points.
3.5.5 Effects of internal Cs\(^+\). In view of the earlier observations that internal Cs\(^+\)-blockade of K\(^+\)-channels prolonged spike duration and blocked the fast AHPs (cf. Fig. 12), the effects of internal Cs\(^+\) applications also were examined with the current- and voltage-clamp techniques. A progressive membrane depolarization (up to 35 mV) and blockade of the AHPs as well as a time-dependent increase in spike duration were observed following penetration of neurons with Cs\(^+\)-containing electrodes (Fig. 18, A and C). During the first 10 min after the initial impalement, there also was a progressive diminution in the transient outward currents (Fig. 18, B and D). A complete blockade of the AHPs and transient outward currents often was observed after 8 min of Cs\(^+\) application. The applications of Cs\(^+\) also blocked other K\(^+\)-currents generating the resting membrane potentials as well as inward rectification, whereas TEA application did not always depolarize neurons and did not block inward rectification. The blocking actions of Cs\(^+\) therefore were more generalized but were similar to the effects of TEA.

3.5.6 Other outward currents. The effects of 4-AP and TEA were examined in 5 neurons by clamping the membrane potentials close to rest and delivering depolarizing voltage commands in order to evoke outward currents. The transient outward currents could be distinguished from other outward currents because of their more rapid decay (Fig. 19). The time course of this decay was similar to that of the tail currents evoked with the hyperpolarizing voltage command paradigm. Application of TEA (5 mM) reduced the envelope size of the outward currents including the transient current (Fig. 19). The application of 4-AP (5 mM) also diminished the envelope of the evoked outward currents, except for faster transient currents (Fig. 19).
Fig. 18. Effects of Cs\(^+\) application on action potentials and transient outward current. The illustrated traces were obtained at specified time intervals following penetration of the neuron. Recordings in B, C and D were obtained during perfusion with a TTX-containing low-[Na\(^+\)] medium. Note the absence of an AHP in C and transient outward current in D.
Fig. 19. Voltage-clamp responses of a neuron to depolarizing command steps. A transient current in addition to other outward currents was evoked from a holding potential of -50 mV. TEA application (at 2 min) blocked the transient current and reduced the overall envelope of currents. Following partial recovery from TEA, 4-AP application (at 3 min) also reduced the current envelope but did not block the transient current. TTX (1 µM) was present throughout.
In 2 neurons where the membrane potential was held at -50 mV, fast transient currents were not observed. Under such conditions, TEA application increased the slope of decay in the outward currents, presumably by blocking more slowly activating currents. On the other hand, 4-AP was a potent blocker of the outward currents evoked at the onset of the voltage commands (Fig. 20).

3.5.7 Combined applications of 4-AP and TEA. The above results indicated that the 4-AP applications blocked outward currents with a fast onset and a relatively slow decay. Therefore, the specificity of 4-AP-actions for the fast-activating, slowly inactivating outward current was assessed by applying 4-AP in combination with 10 mM TEA (n = 4). This procedure permitted suppression of the fast transient current and an improvement of the voltage-clamp. The effects of 4-AP application on the evoked outward currents where neurons were clamped at holding potentials of -70 mV in the presence of TEA and are shown in Fig. 21; here, depolarizing commands elicited a fast-activating, sustained (>1 s) current. Separation of the 4-AP sensitive currents then was obtained by subtraction from the currents observed in the control condition (cf. Fig. 21C).
Fig. 20. Outward currents evoked by depolarizing voltage commands from a holding potential of -50 mV in a neuron that did not exhibit a transient outward current. At 2 min of TEA application the decay of the outward current was increased, presumably due to the blockade of the delayed rectifier. Following a 3.4 min return to control perfusate, a 2 min application of 4-AP did not block the delayed rectifier and produced a large decrease in the outward currents evoked at the onset of the command steps.
control

TEA
5 mM

4-AP
5 mM

1 nA
25 mV

40 ms
Fig. 21. A: currents evoked in the presence of TEA (10 mM) and TTX (1 μM) with voltage commands from a holding potential of -70 mV. B: currents evoked on application of a perfusate containing TEA (10 mM), TTX (1 μM) and 4-AP (5 mM). C: 4-AP-sensitive component of the outward current was obtained by computer subtraction of currents in B and A. Each trace was averaged from two sweeps.
3.6 Studies on the perikaryal invasion of spikes in the TRG

Electrical stimulation of axons was performed in several experiments because this paradigm made it possible to observe the characteristics of the postspike AHPs in the absence of current injection. Secondly, axonal stimulation facilitated the study of the modulation of spike invasion by changes in the perikaryal resting membrane potential. Changes in the membrane potential were induced by injections of DC and/or by application of the K⁺-channel blocker, 4-AP.

3.6.1 Changes in resting membrane potential. The effects of DC injections on the spike characteristics were examined in 6 TRG neurons. Generally, the amplitudes of evoked action potentials were greater at more hyperpolarized membrane potentials. The amplitudes of the AHPs, on the other hand, were greater at more depolarized membrane potentials (Figs. 22 and 23). In four neurons, extrapolated values of the reversal potential for the AHPs averaged -79 ±7 mV S. E. M. In one case, the fast AHPs were followed by afterdepolarizations (ADPs) that had a relatively long time course (Fig. 22). The time course of ADPs was similar to that of the spike afterdepolarizations observed during experiments with Cs⁺ injections (cf. Fig. 13).

Hyper- or depolarization of the membrane with DC injections had marked effects on the invasion of spikes and on the repetitive discharge abilities of the neuronal perikarya. Only small (5-10 mV) depolarizing responses could be evoked with axonal stimulation in 3 neurons that were depolarized by 5-15 mV from rest. On the other hand, hyperpolarization of the resting membrane potentials to levels of -85 to -104 mV did not prevent perikaryal invasion of spikes in these neurons. Also, injections of depolarizing current pulses
Fig. 22. Comparison of responses to axonal and perikaryal stimulation. 
A: responses of a TRG neuron to injection of depolarizing and hyperpolarizing current pulses. A sag was evident in the voltage responses to the two largest hyperpolarizing pulses. B: action potential evoked with stimulation (arrow; 1.9 V, 0.5 ms) of the peripheral axonal field, in the same neuron as in A. Note the fast afterhyperpolarization (AHP) followed by an afterdepolarization (ADP) with a much longer duration. C: action potentials evoked with axonal stimulation at various membrane potentials. Polarization of the membrane was achieved by injection of direct currents. D: A plot of the AHP and ADP amplitudes against the membrane potential. The extrapolated reversal potentials for the AHP and ADP were -85 and -54 mV respectively. Values of AHP and ADP obtained at polarized potentials more positive than -65 mV were not included because spikes evoked at these membrane potentials did not overshoot 0 mV.
evoked action potentials in the perikarya at resting potentials where axonal stimulation failed to elicit a spike. In two neurons, depolarization of the membrane by 15-20 mV did not result in failure of spike invasion. However, depolarization of the membrane led to the induction of repetitive spike discharge following the invasion of a single spike elicited by axonal stimulation (Fig. 23). These observations provided direct evidence that changes in the resting potential affect the ability of axonally generated spikes to invade the perikaryon and that spikes may be generated within the cell body.

3.6.2 Effects of 4-AP. The changes in membrane properties of neurons following applications of 4-AP (1-5 mM) were similar to the effects observed in the absence of axonal stimulation. 4-AP application (1 mM) slightly increased the duration of evoked spikes and also increased the amplitude of the spike overshoot (Fig. 24). Similarly, the amplitude and duration of the spike AHPs was increased, which was at least in part due to the increased input resistance. Electrical stimulation of the axons was more effective in producing spikes in the perikaryon following administration of 4-AP, than during perfusion with the control solution. Another observed event during 4-AP perfusion in two neurons, was the spontaneous appearance of fast (10-15 ms) depolarizations that often reached spike threshold (Fig. 24C).
Fig. 23. Effect of changes in membrane resting potential on the suprathreshold responses evoked by electrical stimulation of axons. Upper traces show the intracellular responses to the brief stimuli (4.3 V; 0.5 ms) indicated by the arrows. The stimulating electrode was positioned in the axonal field distal to the recording electrode. Middle traces correspond to the positive direct current applied through the recording electrode. Response at the left was obtained in the absence of membrane polarization. Note the additional spikes that followed the evoked spike when the resting potential was depolarized to -49 mV.
Fig. 24. Effects of 4-AP on the perikaryal invasion of spikes. A: control responses of a TRG neuron to the extracellular stimulation of the peripheral axonal field. A brief stimulus (3.5 V; 0.5 ms) evoked a local response or an action potential in the perikaryon. B: application of 4-AP (1 mM) led to spike discharge following every stimulus (3.5 V; 0.5 ms). C: in the absence of stimulation spontaneous depolarizing responses and spikes were evident. In A and B upper dashed lines indicate 0 mV and lower dashed lines correspond to the cell membrane potential. Note that the resting membrane potential is maintained at -60 mV with DC injection in all figure traces.
3.7 Studies of autacoid effects in TRG neurons

3.7.1 Bradykinin. Applications of this autacoid in concentrations of 5 to 10 µM for 2-5 min did not produce significant changes in the resting membrane potentials of 4 TRG neurons. Similarly, no change was observed in their input resistances or the characteristics of evoked spikes.

3.7.2 Histamine. Histamine was applied to 9 TRG neurons in concentrations of 10 and 100 µM. In 7 of these neurons, histamine did not significantly affect the subthreshold membrane properties or the characteristics of evoked spikes. However, applications of histamine (100 µM) elicited a large depolarization in two neurons (Fig. 25). The time course and duration of these depolarizations were similar to those observed on perfusion with substance P (see below). The histamine-induced depolarization was accompanied by a decrease in input resistance (75% reduction at peak response). During the depolarizations, injections of depolarizing current pulses elicited spikes that were reduced in amplitude, presumably due excessive inactivation of Na⁺ channels. The depolarization in Fig. 25 was followed by a hyperpolarization to a new resting potential that persisted for 8 min. Subsequent applications of histamine (100 µM) produced smaller (<9 mV) depolarizations in only one of these neurons. Applications of cimetidine (1 mM) for 3 min followed by histamine (100 µM for 1 min) did not block the small depolarizations.

3.7.3 Substance P.

3.7.3.1 Effects on subthreshold membrane properties. The effects of substance P were determined on 50 TRG neurons with resting potentials more negative than -50 mV and spike amplitudes >50 mV. In 35 of these neurons applications of substance P (0.1 µM-10 µM) elicited depolarizing
Fig. 25. Effects of histamine on a TRG neuron.
The resting membrane potential was -58 mV. The large depolarization induced by histamine (100 µM) application in this neuron was followed by a long-lasting hyperpolarization (~8 min) to a new resting membrane potential of -70 mV.
responses such as those illustrated in Fig. 26. The responses were slow in onset, often requiring tens of seconds to develop, large in amplitude (up to 45 mV), and fully reversible on termination of the application. In several neurons, the depolarizations were preceded by small (1-2 mV) hyperpolarizations. In 11 neurons, applications of substance P (2 μM) produced depolarizations that ranged from 5-37 mV with a mean peak amplitude of 14.4 ± 3.4 mV (S. E. M.). The effects could be detected with doses as low as 0.1 μM and were insensitive to the inclusion of TTX (1 μM) in the perfusates. Construction of dose–response relationships was difficult because the neurons varied greatly in their sensitivities to, and frequently became desensitized, after an application of substance P. The "desensitization" was evident from a slow waning of a response despite continued application, and from the reduced responses, or even a total unresponsiveness of a cell, to subsequent administrations of the peptide. Desensitization was long-lasting and apparent even after long recovery periods (e.g., 1 hr). In addition, increases in the responses to the second application of substance P were observed in 5 neurons. Additional applications resulted in comparable or reduced amplitudes of responses.

As illustrated in Fig. 26A, a depolarization evoked by perfusion with substance P was associated with a decrease in input resistance (72%). However, when the peak depolarization was compensated by injecting hyperpolarizing DC, input resistance was increased in 4/4 cells (37 ± 22% S. E. M., range 4-100%) at resting potentials near control levels (Fig. 26B).
Fig. 26. Depolarizing responses to substance P application in 2 cells (A and B). Fast vertical deflections are voltage responses to intracellular injections of constant current pulses (50 ms) monitored in the bottom trace, and represent tests for input resistance. Note that when the membrane potential was returned to the initial resting level using direct current injection (B), the responses to hyperpolarizing test pulses had amplitudes similar to those of control. Supra-threshold responses to depolarizing current pulses are truncated.
Single electrode voltage-clamp analyses in 2 neurons revealed that substance P application produced a large inward shift in the steady-state current (Fig. 27B). However, inward currents evoked by hyperpolarizing voltage commands from a holding potential near rest were increased marginally, and the outward currents that developed on termination of the commands were reduced slightly, during the peak shift in the steady-state current (Fig. 27B).

3.7.3.2 Effects of substance P on spikes and repetitive discharge. Perfusion with substance P also increased the excitability of TRG neurons in other ways. Although "spontaneous" spikes were not initiated, a facilitation of stimulus-evoked spike discharge was observed near the peak of substance P-depolarizations (Fig. 27A). Increases in excitability were detected in 16 of 20 cells that were injected intracellularly with suprathreshold depolarizing current pulses during the depolarizations. In 4 cells where the peptide-induced depolarizations had exceeded 25 mV in amplitude, generation of spikes was impeded, presumably by excessive Na\(^+\)-inactivation.

3.8 Studies on the ionic mechanism of substance P actions
In light of the above observations of substance P effects on TRG neurons, the possible ionic mechanism(s) generating these responses were examined using substitutions of cations in the extracellular medium.

3.8.1 Effects of changes in extracellular [Na\(^+\)]. Perfusion with Na\(^+\)-deficient solution produced membrane hyperpolarizations in 8/10 neurons (cf. section on membrane potential dependence on [Na\(^+\)].)
Fig. 27. A: substance P application (10 μM) facilitates development of repetitive spikes. Repetitive discharge was evoked with depolarizing current injection near the peak of a substance P-induced depolarization, but could not be elicited in control conditions. Dashed lines indicate 0 mV. B: voltage clamped response of the same cell to a second application of substance P (10 μM). A large inward shift (right bottom) in the steady-state current was observed. Note also the slight increase in the evoked inward current and the reduction in the outward currents.
Fig. 28. Reduction of the response to substance P (3 μM) in a neuron by perfusion with a Na\(^+\)-deficient solution. Resting potentials were -53, -54, and -53 mV in control, low-[Na\(^+\)], and recovery conditions, respectively. Spikes recorded in control ACSF had amplitudes of ~85 mV and were blocked in the Na\(^+\)-deficient medium (not shown). Ten minutes were allowed for recovery prior to each application of the peptide.
control
substance P

low-\(\text{Na}^+\)
substance P

recovery
substance P
Stimulus-evoked spikes were abolished in conditions of low-Na\(^+\) perfusate (cf. Fig. 4). When substance P was applied in the low-Na\(^+\) perfusate, the depolarizing responses were reduced or blocked in 3/4 neurons (Fig. 28). These data indicated that the substance P effects depend partly on the presence of extracellular Na\(^+\).

3.8.2 Effects of changes in extracellular [Mg\(^{2+}\)]. The effects of removing Mg\(^{2+}\) from the extracellular medium were examined in 5 neurons. The resting membrane potential was not significantly affected by perfusion with a Mg\(^{2+}\)-deficient solution. The input resistance also was not affected. No obvious changes were observed in the action potentials evoked by current pulse injections in the absence of Mg\(^{2+}\).

The depolarizing responses to substance P were greatly reduced in Mg\(^{2+}\)-deficient conditions. Fig. 29 shows chart recorder traces of recordings obtained from a neuron in which the response to substance P in a low-[Mg\(^{2+}\)] solution was reduced to ~25 of the peak response obtained in the control perfusate. In other neurons similarly tested, the responses to substance P application were completely and reversibly abolished in Mg\(^{2+}\)-deficient solutions (n = 4).

3.8.3 Effects of Ca\(^{2+}\)-channel blockade. The low-Ca\(^{2+}\), Co\(^{2+}\)-containing perfusates were inconsistent in their ability to affect, significantly, the responses to substance P application. This was in contrast to the readily observed effects of Na\(^+\)- or Mg\(^{2+}\)-removal on the substance P-induced depolarizations. In 2 neurons, decreases in the peak amplitude of substance P-induced depolarizations were observed in low-Ca\(^{2+}\), Co\(^{2+}\)-containing perfusates, whereas in 3 other neurons increases in the responses were evident.
Fig. 29. Reduction in the response to substance P (2 μM) in a TRG neuron by perfusion with a Mg$^{2+}$-deficient solution. Resting membrane potential was -75 mV during control conditions. Spontaneous cell depolarization that followed recovery from the control application of substance P required injection of hyperpolarizing direct current to maintain resting potential at -75 mV. Ten minutes were allowed for recovery prior to each application of the peptide. Action potentials were not affected by the Mg$^{2+}$-deficient perfusate.
3.8.4 Voltage-clamp studies of substance P actions in the presence of K⁺-channel blockers. The results of Na⁺-substitution experiments are consistent with the possibility that Na⁺-influx was involved in the depolarizing responses to substance P. Therefore, the effects of substance P applications (2 µM) were examined in the presence of K⁺-channel blockers, with voltage-clamp analysis techniques.

Combined applications of 4-AP (5 mM) and TEA (10 mM) produced large increases in the input resistance which allowed an improvement in the voltage clamp. Also, it was reasoned that the blockade of certain K⁺-conductances that may be involved in the responses to substance P would allow to separate the peptide-induced activation of a proposed inward current. Two effects of substance P were observed following its application (2 µM) to neurons that were clamped near resting potentials in the presence of K⁺-channel blockers (n = 2). First, there was an inward shift in the steady-state current (Fig. 30) that was similar to the steady-state current shifts observed with substance P applications in the absence of K⁺-channel blockers (cf. Fig. 27). Secondly, an increase in the inward currents evoked by hyperpolarizing voltage commands (Fig. 30A and B) could be observed from the current-voltage relationships obtained near the peak shift in the steady-state current. These results provided additional evidence that substance P-induced responses may be due in part to the activation of an inward current.
Fig. 30. Effects of substance P application during K⁺-channel blockade. A: substance P evoked an inward current in the presence of K⁺-channel blockers. The responses to substance P were obtained during the peak change in the steady-state current. The membrane potential was clamped at -60 mV. TEA (10 mM) and 4-AP (5 mM) were present throughout. B: current-voltage relationship for the responses exemplified in (A). The measurements were made at ~10 ms following the onset of the voltage commands. The lines (1st-order regression) through the points were computer generated. The regression line for the recovery was the same as for control and therefore omitted for clarity.
A

control substance P recovery

B

Voltage (mV)

Current (nA)

-50 -40 -30 -20 -10 0 0.0 0.5 1.0 1.5

● control ○ substance P
4 DISCUSSION

These investigations have revealed many significant features in the actions of the selective K⁺-channel blockers -- TEA, 4-AP and Cs⁺, as well as certain autacoids, on the membrane properties of TRG neurons. Because the main purpose of these studies was to determine the ionic mechanisms in the excitability of TRG neurons, particularly those mediated by K⁺, the most pertinent findings in the investigations will be summarized according to this theme before commencing a general discussion in the context of signal transmission within sensory ganglia.

The resting membrane potential of TRG neurons was found to be dependent on extracellular [K⁺] and to a lesser extent on extracellular [Na⁺] and [Ca²⁺]. Injections of subthreshold intracellular current pulses evoked various voltage- and time-dependent responses such as membrane potential oscillations and sags in the voltage responses to hyperpolarizing current pulses. Suprathreshold current pulses evoked spikes that could be differentiated into two groups, based on their repolarization phase characteristics. Differences in the sensitivities of spikes to Na⁺-channel blockade were demonstrated with applications of tetrodotoxin (1 µM), a specific channel blocker.

The sub- and suprathreshold membrane responses to current pulses were greatly affected by the three K⁺-channel blockers -- TEA, 4-AP and Cs⁺. The membrane of TRG neurons was depolarized following applications of each of the above agents, internal Cs⁺ being the most effective. External applications of TEA (5-10 mM) produced a reduction in the spike afterhyperpolarizations and also decreased the repetitive discharge abilities of neurons. The latter effect was evident despite increases in input
resistance, depolarization, decreased spike threshold and enhanced spike amplitude. In contrast, applications of 4-AP (0.5-5 mM) did not block the AHPs and greatly increased the repetitive discharge ability of TRG neurons, sometimes resulting in spontaneous discharge of spikes. Combined applications of 4-AP (1-5 mM) and TEA (5-10 mM) evoked long-duration spikes that were insensitive to TTX applications, but were blocked in low-[Ca$^{2+}$], Co$^{2+}$-containing perfusates. Such spikes were followed by prolonged AHPs. In the experiments performed with Cs$^+$-containing electrodes, prolonged AHPs were not observed; indeed, spikes were followed by afterdepolarizations. These spikes and afterdepolarizations also were blocked in Ca$^{2+}$-deficient solutions that contained Co$^{2+}$, a Ca$^{2+}$-channel blocker.

Voltage-clamp analyses revealed that a transient outward current in TRG neurons was susceptible to blockade with TEA in a dose-dependent manner and was not affected by applications of ACh and other muscarinic agents. This current also was little affected by 4-AP applications. However, such applications were effective in blocking another outward current that had a relatively long time course of inactivation. Because this current could not be blocked by high doses of TEA, another distinct K$^+$-channel is inferred.

Applications of 4-AP enhanced the ability of spikes evoked by electrical stimulation of the axons to invade the perikaryon. In addition, spontaneous fast depolarizations that often reached spike threshold were observed following 4-AP applications, suggesting that these phenomena may result in the generation of spontaneous discharges within perikarya of TRG neurons.

Experiments using electrical stimulation of axons also showed that hyperpolarization of the resting membrane potential did not prevent the invasion of spikes into the perikaryon. However, perikaryal membrane depolarization could result in the failure of spike invasion, or in other
cases, in the generation of additional spikes following the invasion of a single spike in the perikaryon.

Studies on the actions of autacoids in TRG neurons demonstrated that bradykinin (5-10 μM) or histamine (100 μM) applications did not significantly affect the electrical membrane properties in most neurons, although large membrane depolarizations were evoked by histamine in 2 of 9 neurons. Subsequent applications of histamine to these neurons evoked only small membrane depolarizations, possibly attributable to desensitization at H1-receptors since they could not be abolished with application of cimetidine, an H2-receptor antagonist.

The majority of neurons (35 of 50) exhibited reversible depolarization following application of substance P (0.1-10 μM). Repetitive spike discharge ability was enhanced during such depolarizations, except when the amplitude of an evoked depolarization exceeded 25 mV. Substance P-induced depolarizations were accompanied by decreases in input resistance. However, when the peak depolarization was compensated by hyperpolarizing DC-injections, input resistance was increased, suggesting that a net blockade of conductances was involved in the responses to substance P. Voltage-clamp analyses of the substance P effects revealed a large inward shift in the steady-state current at holding potentials near rest. Also, the inward currents evoked by voltage command steps were increased slightly, whereas the outward currents evoked on termination of the voltage commands were reduced, during the time of peak responses to substance P application. Voltage-clamp studies in the presence of 4-AP (1 mM) and TEA (10 mM), provided additional evidence that substance P applications evoked an inward current in addition to blockade of a conductance, as inferred from input resistance increases observed during current-clamp experiments. The substance P-induced depolarizations were
greatly reduced in conditions of lowered extracellular $[\text{Na}^+]$ and $[\text{Mg}^{2+}]$ and were not affected significantly during perfusion with low-$[\text{Ca}^{2+}]$, Co$^{2+}$-containing solutions.

These data suggest that substance P may produce its excitatory effects in TRG neurons by decreasing the resting conductance of ions, possibly $\text{K}^+$, concomitantly with an increase in conductance to other ions, possibly, $\text{Na}^+$.  

4.1 **Membrane potential dependence on extracellular cations**

The results of these experiments employing changes in extracellular $[\text{K}^+]$ indicate that TRG neurons are similar to other neurons in the CNS and PNS. Specifically, their resting membrane potentials are dependent on external $[\text{K}^+]$. The deviation of the experimentally derived fit to the points in Fig. 3 from the straight line expected for a single ionic species ($\text{K}^+$), suggests that other ions as well as $\text{K}^+$ contribute to the resting potentials. This suggestion is corroborated by results of the experiments using $\text{Na}^+$-substitution and $\text{Ca}^{2+}$-channel blockade by incorporation of Co$^{2+}$ in the extracellular media. During perfusion, hyperpolarization was observed consistently, inferring that $\text{Na}^+$ and/or $\text{Ca}^{2+}$ contribute significantly to the resting membrane potential of TRG neurons. Indeed, influx of $\text{Na}^+$ and/or $\text{Ca}^{2+}$ may occur through channels that are susceptible to blockade with Co$^{2+}$ (Konnerth, 1987).

4.2 **Electrical membrane properties of TRG neurons**

In general, the electrical properties of TRG neurons appear to be similar to those of other cerebrospinal ganglion cells, particularly DRG neurons. The offstream anatomical position of neuronal perikarya with respect to their axonal processes has led to the view that the cell bodies are of rather limited electrophysiological significance for primary afferent transmission in the intact animal (Lieberman, 1976). However, certain membrane properties
of TRG neurons, to be discussed below, are consistent with more interesting interpretations of the functions of their perikarya within the trigeminal sensory system. Also, since it is not yet possible to record intracellularly from the central (or peripheral) terminations of TRG fibers, these results on TRG perikarya may be a preview of membrane electrical and pharmacological properties of their terminations (cf. Feltz and Rasminsky, 1974; Deschenes et al., 1976).

4.2.1 Subthreshold responses. The TRG neurons in these investigations exhibited time-dependent rectification in their hyperpolarizing voltage responses to intracellular injections of current pulses; this has been described in frog DRG neurons (Ito, 1957), and later was suggested to be a consequence of removal of partial Na\(^+\)- and/or Ca\(^{2+}\)-channel inactivation (Czeh et al., 1977). Mayer and Westbrook (1983), who performed voltage-clamp analyses on cultured mouse DRG neurons, found that this form of rectification was a consequence of "mixed" Na\(^+\)- and K\(^+\)-currents. An involvement of external Na\(^+\), possibly mediating such currents, also is supported by our observations that the rectification is reduced in low-Na\(^+\) solutions, or by applications of tetrodotoxin. In addition, complete elimination of the rectification has been observed in Na\(^+\)-free solution, with tetrodotoxin present (Gallego, 1983). A reduction in Na\(^+\)-inactivation as well as a presumed inward Na\(^+\)-current that is turned on by the induced hyperpolarization, thereby would contribute to the anodal break response on termination of the hyperpolarizing current pulse (cf. Fig. 5). The voltage responses of TRG neurons to intracellular injections of depolarizing current pulses usually were characterized by damped oscillations that were unaffected by prolonged applications of tetrodotoxin, indicating that a TTX-sensitive, Na\(^+\)-conductance was not involved in the subthreshold membrane oscillations.
4.2.2 Action potentials. In these, as in previous investigations (Spigelman, 1986; Puil and Spigelman, 1988), two types of action potentials could be distinguished on the basis of an absence or presence of a plateau (hump) on the falling phase of evoked spikes. Although we were not able to identify electrophysiologically different types of TRG neurons for technical reasons, DRG cells that discharge humped spikes of long duration have been classified as slowly conducting, unmyelinated C-neurons and myelinated Aδ-neurons which transmit nociceptive information (Czeh et al., 1977; Görke and Pierau, 1980; Harper and Lawson, 1985; Rose et al., 1986). An influx of Ca\(^{2+}\) ions, in addition to Na\(^{+}\) ions, may participate in the genesis of long duration spikes in ganglionic neurons (Ito, 1982). For example, the Na\(^{+}\)-conductance in such humped spikes is only partly sensitive to blockade with TTX, whereas fast spikes without a hump are blocked completely by TTX administration (Gallego, 1983; Stansfeld and Wallis, 1985; Yoshida et al., 1978). The present results on TRG neurons suggest that a main component in the development of either type of action potential is a Na\(^{+}\)-current which, in some cases, is TTX-insensitive. Because the falling phase, particularly the later part of TRG action potentials including the fast afterhyperpolarization, could be blocked with either external TEA or internal Cs\(^{+}\) application, K\(^{+}\) probably contributes to most of the repolarization phase of humped and non-humped spikes.

4.2.3 Postspike afterhyperpolarizations. Although the peak amplitudes of AHPs of TRG neurons were similar to those observed in other mammalian sensory neurons, the durations were longer (Gallego and Eyzaguirre, 1978; Görke and Pierau, 1980; Holz et al., 1985; Stansfeld and Wallis, 1985). In addition, the AHPs of TRG neurons that exhibited humped spikes were larger in amplitude and longer in duration than the AHPs of sensory neurons
with short spike durations. In the DRG of the pigeon (Görke and Pierau, 1980) and the frog (Holz et al., 1985), action potentials of identified C-neurons are accompanied by AHPs with amplitudes and durations that exceed the AHPs of A-neurons. In the rabbit nodose ganglion, the peak amplitudes of the AHPs are similar in both C- and A-neurons, but the AHP duration is much longer in the case of C-neurons (Stansfeld and Wallis, 1985). Persistent afterhyperpolarizations lasting several hundreds of milliseconds or seconds in duration like those observed in nodose ganglion neurons (Jaffe and Sampson, 1976) were not commonly observed following the repetitive discharge evoked by current pulse injection into TRG neurons. The small long-lasting (<150 ms) AHPs may be a result of a Ca$^{2+}$-activated K$^+$-conductance activated by the repetitive discharge in TRG neurons as in other sensory neurons (Weinreich, 1986). However, the fast AHPs accompanying humped and non-humped spikes in TRG neurons are likely to be a consequence of activation of a K$^+$-conductance, as inferred from the AHP blockade by external TEA or internal Cs$^+$ applications.

4.3 Membrane electrical responses of human sympathetic neurons

Although human ganglia have been employed as a source of primary cell cultures (Scott et al., 1979; Fukuda et al., 1983), these investigations have shown that slice preparations of sympathetic ganglia of peripherally perfused, brain-dead humans can be kept viable under in vitro conditions for more than 12 hr. A well-known clinical observation is that, during lower limb surgical procedures that involve an aortic clamp, an interruption of blood supply to the ganglia for several hours is not associated with clinical sequelae. This apparent resistance to anoxia could not be confirmed experimentally in slices of ganglia that had been kept in cold, oxygenated ACSF.
for ~8 hr, or in similar preparations of human trigeminal root ganglia excised 4 hr postmortem, in the present investigations. However, intracellular recordings could be obtained from neurons in *in vitro* slice preparations of acutely excised human sympathetic ganglia, for electrophysiological analyses of membrane properties and pharmacology.

The resting membrane potentials and average value of input resistance (~29 MΩ) in the human neurons were mostly in the same ranges reported for various lumbar sympathetic neurons in guinea pigs and cats (cf. Skok, 1973). The wide variation of input resistance values in human neurons probably reflects differences in the diameters of the perikarya, as well as electrode sampling bias, particularly since successful recording depends on the penetrability of the strong, fibrous connective tissue surrounding the cells. When neurons have been isolated from human sympathetic ganglia prior to tissue culture, their diameters have been found to be ~40-60 μm (Fukuda et al., 1983). The above data and the long membrane time constants measured in 3 neurons are consistent with certain remarkable properties of sympathetic neurons in other mammals. For example, the high input resistance values and low threshold for spikes generated on current injections into sympathetic neurons of rodents have been attributed to a high membrane (specific) resistance rather than to their size (cf. Perri et al., 1970).

An interesting result here was the frequent appearance of TTX-sensitive spikes on the anodal break responses as well as the small outward tail currents revealed by the voltage step commands. These responses, like those observed in rat sympathetic neurons (Galvan, 1982), suggest that an outward current may modulate neuronal spike genesis during the depolarizing rebound of the anodal break response.
In general, the pharmacological sensitivities of the spikes of human neurons appear to be similar to sympathetic neurons in other mammals (cf. McAfee and Yarowsky, 1979). The observed alterations in spike amplitude and duration in human neurons during TEA applications suggest blockade of a voltage-sensitive $K^+$-conductance. On the other hand, the postspike AHPs were slightly reduced by the TEA applications indicating the existence of a distinct $K^+$-conductance which also was unaffected by 4-AP applications.

4.4 Differences in the actions of $K^+$-channel blockers on TRG neurons

The observations that applications of 4-AP had excitatory effects in TRG neurons in contrast to the ineffectiveness of TEA applications, suggest separate sites of blocking action on the outward currents. Presumably, the amphipathic property of 4-AP confers ready access of 4-AP molecules to the $K^+$-channel sites within the neuronal membranes, whereas the more hydrophilic TEA is likely to gain access to the blocking sites from the external membrane surface i.e., from the aqueous phase (Thompson and Aldrich, 1980). An important difference observed in their actions on TRG neurons was the greater efficacy of TEA in blocking the transient outward currents whereas outward currents that had much longer time courses of inactivation were much more readily blocked by 4-AP applications.

The actions of another $K^+$-channel blocker, internal $Cs^+$, on spikes and the transient outward current were similar to those of TEA. However, unlike 4-AP and/or TEA, $Cs^+$ injections produced a greater degree of blockade of the conductances that presumably contribute to the resting membrane potential. In addition, $Cs^+$ blocked the afterhyperpolarizations observed during spontaneous or stimulus-evoked spiking in the presence of 4-AP and TEA. Indeed, prolonged afterdepolarizations were associated with spikes
during internal Cs\(^+\) applications. These probably were a consequence of an activation of a Cl\(^-\)-current. For example, the intracellular [Cl\(^-\)] in DRG neurons is higher than the extracellular [Cl\(^-\)] (Nishi et al., 1974). In TRG neurons, \(\gamma\)-aminobutyrate evokes depolarizations during intracellular recording with \(K_2SO_4\)-containing electrodes and these are susceptible to blockade with bicuculline (Spigelman, 1986; Puil and Spigelman, 1988). The present observations of spike afterdepolarizations obtained with \(Cs_2SO_4\)-filled electrodes provide further support for the suggestion of a high intracellular content of Cl\(^-\) in TRG neurons.

Ca\(^{2+}\)-activated Cl\(^-\)-conductance has been described in cultures of DRG (Mayer, 1985) and spinal cord neurons (Owen et al., 1984). In the present studies, perfusion with low-[Ca\(^{2+}\)], Co\(^{2+}\)-containing media abolished the Ca\(^{2+}\)-spikes and the afterdepolarizations. These observations are consistent with the presence of a Ca\(^{2+}\)-activated Cl\(^-\)-conductance in TRG neurons. A likely physiological role for an inward current mediated by Cl\(^-\) would be to reduce membrane excitability by a shunting action. Assuming similar currents exist in the terminals of sensory neurons, activation of a Cl\(^-\)-conductance also may serve to limit transmitter release, as proposed previously in the mechanism of presynaptic inhibition in the CNS (Eccles, 1964; Nicoll and Alger, 1980; Padjen and Hashiguchi, 1983).

4.4.1 Membrane potential oscillations. The tendency for the resting potentials of TRG neurons to oscillate on depolarization with subthreshold amounts of intracellularly-injected current pulses was suppressed during TEA applications. The TEA-actions were accompanied by a reduction in the repetitive spike discharge that could be evoked by depolarizing current injections. The oscillations observed at resting potential levels that were subthreshold for spike genesis, are likely to be a consequence of the same
membrane properties giving rise to the resonant behavior in the impedance magnitude functions of TRG neurons (Puil et al., 1987, 1988; Puil and Spigelman, 1988).

The importance of this behavior or its ionic generation in the entrainment of neuronal discharge is related particularly to the observations that excitable cells are most likely to develop a repetitive discharge in response to depolarizing inputs at the natural frequency of oscillation in the membrane, i.e., at potentials where the resonant behavior becomes prominent in the impedance magnitude function (Clapham and DeFelice, 1976, 1982; Puil et al., 1987). Therefore, the findings that TEA blocks resonance (Puil et al., 1988) and the oscillations of membrane potential as well as the tendency in some TRG neurons to discharge repetitive spikes, take on special significance in view of the other concomitant effects of TEA. The small depolarization induced by TEA, the reduction of spike threshold which was at least partly due to a TEA-evoked increase in input resistance, and blockade of postspike afterhyperpolarization, would represent favorable conditions for repetitive spike genesis. These observations suggest that the $K^+$-conductance(s) associated with oscillations of the membrane potential may modulate the level of $Na^+$-inactivation in TRG neurons.

The above effects of an agent which blocks various $K^+$-channels to varying degrees (Armstrong and Hille, 1972) suggest that more than one type of $K^+$-conductance may be involved in the excitabilities of TRG neurons. Because several neurons were only slightly depolarized by TEA applications in these experiments, the strong reduction of oscillatory behavior cannot be explained by the observed changes in the resting potentials (cf. Fig. 8A). The simplest explanation for the depression is that TEA blocks the time-
dependent $K^+$-conductances (Armstrong and Hille, 1972) which are manifest as oscillations in the time-domain and as resonance in the frequency-domain (Puil et al., 1988).

An interesting feature of 4-AP actions on most TRG perikarya is the enhancement of their subthreshold oscillations which may lead to the development of repetitive spike firing. This feature is opposite to that observed with TEA administrations to TRG neurons, but was evident with TEA administrations to spinal root axons in the rat (Baker et al., 1987). The oscillations which also were induced by 4-AP application and blocked when TEA was additionally applied, were presumably unmasked by the 4-AP blockade of a slow outward current. Each oscillation probably results from a fast, TTX-insensitive inward current and a repolarizing outward $K^+$-current that is sensitive to blockade by TEA. If the inward current (in the presence of 4-AP) were carried partly by $Ca^{2+}$ (cf. Rogawski and Barker, 1983), the slow depolarizing shift on which the oscillations are superimposed would be generated by intracellular $Ca^{2+}$-accumulation and/or activation of an outward $Cl^-$-current (Mayer, 1985). On reaching threshold, these oscillations could facilitate repetitive spike discharge and give rise to a burst pattern.

4.4.2 Repetitive spike firing. Usually, most TRG neurons in situ are electrically silent (cf. INTRODUCTION), presumably because of an absence of synaptic excitation (cf. Lieberman, 1976) and as a result of various voltage- and time-dependent outward currents such as those observed during voltage-clamp experiments. These currents are active near resting potentials of $\sim$-60 to -70 mV. Indeed, many TRG neurons do not discharge repetitively, i.e., more than one spike, during intracellular injections of suprathreshold step currents (cf. Fig. 8B). In the presence of 4-AP this situation
changes, and with imposed depolarizing step currents, continuous repetitive firing may be observed in TRG neurons. Similar phenomena have been observed after 4-AP application to neurons of sympathetic ganglia (Galvan and Sedlemeir, 1984) and dorsal root axons (Kocsis et al., 1986) or after TEA application to spinal root myelinated axons (Baker et al., 1987).

The facilitation of firing by 4-AP may be a result of blockade of a slow $K^+$-current that produces a decrease in spike accommodation. Note that in the control conditions for the cell of Fig. 8B, only a single spike could be evoked with a range of suprathreshold depolarizing current stimuli. Indeed, subsequent experiments show that a current with such characteristics exists in TRG neurons.

The observations of spontaneous fast depolarizations that occur during applications of 4-AP in some TRG neurons suggest that such depolarizations may initiate the spontaneous repetitive spike discharge in sensory neurons. These responses may result from spike activities in the axon and be detected in the impaled perikaryon only after increases in membrane resistance were induced by 4-AP. An alternative hypothesis is that 4-AP may activate an inward current that produces the transient membrane depolarizations. The latter possibility is supported by the observed enhancement of Ca$^{2+}$-currents by 4-AP in spinal neurons (Rogawski and Barker, 1983).

4.5 **Comparison of transient outward current $[I_{(T)}]$ with $I_A$**

The TEA-sensitive transient current observed in TRG neurons has kinetics that are qualitatively similar to those of the transient A-current ($I_A$) in central and sympathetic neurons of mammals. A major difference is that the inactivation of the current in TRG neurons can be reduced with relatively small hyperpolarizing voltage commands (cf. Figs. 14A and 15B). In some TRG
neurons, this probably accounts for the ease with which the transient outward current may be activated by depolarizing voltage commands from holding potentials near rest (e.g. -50 to -60 mV). Despite some similarity of this TEA-sensitive current in TRG neurons to $I_A$ in other neurons, the two currents are most likely mediated by ionic channels in the membrane that have dissimilar infrastructures. Because of these distinctions in sensitivities to 4-AP and TEA applications, as well as a possible coexistence of a 4-AP-sensitive $I_A$ observed in many other types of neurons in vertebrates and invertebrates, the TEA-sensitive outward transient current in TRG neurons will be referred to as $I_{(T)}$.

The applications of muscarinic agonists did not affect the resting membrane properties or $I_{(T)}$. The ineffectiveness suggests an absence of receptors for acetylcholine on the perikarya of TRG neurons, as found in other primary sensory neurons (cf. Lieberman, 1976), although nodose neurons in culture can be depolarized by acetylcholine applications (Baccaglini and Cooper, 1982).

4.5.1 Ionic species mediating $I_{(T)}$. The main ionic species involved in $I_{(T)}$ is likely $K^+$, whereas the current has only a small dependency on external $Ca^{2+}$. The slight reduction in $I_{(T)}$ that was observed during perfusion with low-$[Ca^{2+}]$, $Co^{2+}$-containing solutions was similar to the modest diminution in the transient outward currents observed during such conditions in nodose ganglion neurons (Stansfeld et al., 1986). These dependencies on $Ca^{2+}$ in TRG and nodose neurons are intermediate to those of $I_A$ of mammalian sympathetic ganglion neurons where Galvan and Sedlmeyer (1984) have reported almost complete blockade of $I_A$ by $Cd^{2+}$ and $Mn^{2+}$ applications, whereas an insensitivity of $I_A$ to $Cd^{2+}$ applications was observed by Belluzzi et al. (1985).
In TRG neurons, perfusion with the low-[Ca\textsuperscript{2+}]- or low-[Na\textsuperscript{+}]-containing solutions also produced an outward shift in the steady-state currents, suggesting that Na\textsuperscript{+} and Ca\textsuperscript{2+} contribute significantly to the steady state currents in TRG neurons at rest. A Na\textsuperscript{+}-current mediated by proton-transformed Ca\textsuperscript{2+}-channels can be blocked in dorsal root ganglion (DRG) neurons by applications of Cd\textsuperscript{2+}, Co\textsuperscript{2+} or certain other divalent cations (Konnerth et al., 1986). Indeed, proton-sensitive Na\textsuperscript{+}-currents have been demonstrated in cultured TRG neurons (Krishtal and Pidoplichko, 1980). However, it is unlikely that the outward shift or I(\text{T}) is mediated by Na\textsuperscript{+}-dependent, K\textsuperscript{+}-channels similar to those observed in cultured TRG neurons because of their differing kinetics of activation as well as the sensitivity of these channels in cultured neurons to blockade with TTX (Bader et al., 1985).

4.5.2 Membrane repolarization. The observations that internal Cs\textsuperscript{+} applications blocked both I(\text{T}) and the AHPs in TRG neurons suggest that I(\text{T}) contributes to the slow repolarization of TRG neuronal membranes, at least in the subthreshold regions depolarized from the initial resting state. Although the time course of I(\text{T}) activation cannot be determined with single electrode voltage-clamp techniques, this current may contribute partly to the genesis of AHPs which have similar time courses. The current generating the AHP is well-suited temporally to influence repetitive spike discharge by indirect actions on the voltage dependent Na\textsuperscript{+}-channels. A steady-state inactivation of the TTX-sensitive Na\textsuperscript{+}-current is half-maximal at ~ -80 mV, and complete at -40 mV in rat sensory neurons (Kostyuk, 1981). In this resting potential range, the AHPs would remove Na\textsuperscript{+} inactivation
thereby allowing the reactivation of voltage-dependent Na\textsuperscript{+}-channels and promoting spike discharge. In this scheme, the blocking actions of TEA (on the generation of I\textsubscript{(T)} and the AHPs) would inhibit repetitive discharge.

An involvement of I\textsubscript{A} in spike repolarization has been demonstrated in sympathetic ganglion neurons (Belluzzi et al., 1985). Since the kinetics of I\textsubscript{(T)} activation are unknown, it would be premature to exclude a contribution of I\textsubscript{(T)} to the mechanism of spike repolarization in TRG neurons (cf. Bader et al., 1985). This reservation receives emphasis from the observations of a slight blockade of I\textsubscript{(T)} during administration of 4-AP; such applications produce small increases in the durations of directly evoked spikes, suggesting a coexistence of an I\textsubscript{A} in TRG neurons.

4.5.3 Other outward currents. These investigations have demonstrated that 4-AP applications blocked an outward current that had a fast onset and a relatively slow time course of inactivation. The combined applications of 4-AP and TEA allowed a separation of this current from I\textsubscript{(T)} as well as the TEA-sensitive delayed rectifier current.

Although the fast onset of the 4-AP-sensitive current is indicative of its participation in spike repolarization, this current may be better suited than I\textsubscript{(T)} to exert a steady influence on the repetitive discharge capabilities of TRG neurons. The rather long time course of inactivation suggests an explanation for the general ineffectiveness of current pulse injections to evoke repetitive spike discharge in a majority of TRG neurons in these and previous studies (Spigelman, 1986). The stabilizing influence of the fast-activating, slowly-inactivating current is removed by 4-AP application and this allows repetitive firing to occur.
The 4-AP-sensitive outward current in TRG neurons resembles the fast, sustained current in nodose ganglion neurons which is blocked by 4-AP, and dendrotoxin (Stansfeld et al., 1986; 1987), as well as the non-inactivating K\(^+\)-current in DRG neurons which is blocked by β-bungarotoxin (Peterson et al., 1986). This pharmacological similarity (i.e., sensitivity to 4-AP) may be common to primary sensory neurons. In contrast to other neurons, the putative \(I_A\) in nodose neurons is not affected by 4-AP or TEA administration (Stansfeld et al., 1986). Another difference is that both \(I(T)\) and the fast, sustained current can be observed in the same TRG neurons, whereas the analogous currents are present in separate A- and C-populations of nodose neurons.

4.5.4 Significance. The duration of action potentials in rat primary afferent axons can be increased by 4-AP, but not by TEA applications (Grafe et al., 1985; Kocsis et al., 1987). The present investigations demonstrated only minor effects of 4-AP applications on spike shape compared to TEA, but a pronounced enhancement of repetitive spike firing was observed; these effects were opposite to those obtained with TEA applications.

The findings in TRG neurons raise an intriguing question about the physiological significance of differences in pharmacological sensitivities of K\(^+\)-channels in sensory myelinated axons and perikarya of mammals. Although early investigations of mammalian myelinated axons indicated an absence of K-currents at the nodes (Horackova et al., 1968; Chiu et al., 1979; Brismar, 1980), at least two pharmacologically separable K\(^+\)-currents have been subsequently described (Grafe et al., 1985; Baker et al., 1987). These currents are more commonly observed in the axons of younger animals and also are present at the internodal segments of demyelinated fibers (Chiu and Ritchie, 1980). The perikaryal membranes of primary sensory neurons
exhibit far greater diversity of outward currents in a comparison to their axons. It seems unlikely that these currents are simply a vestigial representation of ontogeny. Indeed, they may have important functions in the adult sensory nervous system.

Perikaryal outward currents, such as the 4-AP-sensitive sustained outward current in TRG neurons, provide a plausible mechanism for the prevention of excessive spike discharge in trigeminal sensory transmission. In circumstances where this braking influence has been removed, somatic invasion by a single action potential may initiate spike discharge in bursts (cf. Fig. 23) which would be propagated to the central and peripheral terminations. The generation of somatic spike bursts in the TRG may occur in certain conditions such as trigeminal neuralgia where a brief sensory stimulus can induce paroxysmal attacks of pain. Also, certain conditions may cause a release of neuroactive autacoids (e.g. histamine, substance P) in the TRG that would affect, either directly or indirectly, the excitabilities of perikarya.

4.5.5 Bursts and ionic mechanisms. The 4-AP induced train of repetitive activity in the cell of Fig. 10B could be transformed into a recurrent burst pattern by imposed hyperpolarization to a background potential where a slow outward current may have developed [cf. successive increase in the amplitudes of the spike afterhyperpolarizations in Fig. 10B, right].

A second type of burst (or modification of the first type due to increase in Ca\(^{2+}\)-influx) was evident with a combined application of 4-AP and TEA (cf. Fig. 10C). These "spontaneous" bursts of action potentials were observed in several TRG neurons and are similar to those reported in neurons of olfactory cortical slice preparations (Galvan et al., 1982) and in peripheral sensory axons (Kocsis et al., 1987) after application of
4-AP. Here, one may presume that a fast inward current sensitive to blockade with TTX produced the spikes and a much more slowly activated inward current, possibly due to unopposed, or 4-AP enhanced (Rogawski and Barker, 1983), Ca$^{2+}$-influx gave rise to the background depolarization of the spike burst. The Na$^+$-inactivation and a slower repolarizing current, insensitive to blockade with 4-AP or TEA, presumably reduced the spike amplitudes and terminated each burst. This termination may be a direct result of a Ca$^{2+}$-dependent, outward K$^+$-current, as suggested by the cardiac-like spikes that had prolonged AHPs when the neuron was hyperpolarized by DC-current injection (Fig. 10C; cf. Heyer and Macdonald, 1982; Calabresi et al., 1987). The prolonged AHPs that were observed following Ca$^{2+}$-spikes (Fig. 13A) provide further support for this suggestion.

4.5.6 Significance. The tendencies of TRG neurons to discharge in bursts, given an appropriate stimulus such as a small abrupt depolarization, are a consequence of membrane characteristics that manifest as oscillations in the responses to injected depolarizing current pulses. Under physiological conditions, such stimuli may include electrical impulse activity from the trigeminal axons that invades the TRG perikarya and, slow depolarizing shifts in their membrane potentials as a result of the actions of a chemical mediator such as substance P, in the TRG. This complex electrical behavior of the TRG perikarya would likely modulate transmission of afferent activity along the trigeminal nerve.

It is possible that the above tendencies of TRG neurons may be exaggerated in pathophysiological conditions such as trigeminal neuralgia (or paresthesias, cf. Kocsis et al., 1986). For example, continuous spike firing including transformation into bursts can occur when there is an interference with, or a defect in K$^+$-channel function, as observed in the
present experiments with 4-AP applications to the TRG neurons. Further impairment of the K\(^+\)-channel system, as in the combined blockade by 4-AP and TEA application, may result in the second type of burst activity (cf. above section on *Bursts and ionic mechanisms*).

4.6 **Spike initiation in the TRG**

Previous electrophysiological investigations of sensory neurons in several species have revealed that action potentials travelling in an orthodromic direction always invade the neuronal perikaryon (cf. Lieberman, 1976). The present investigations in guinea pig TRG neurons showed that changes in resting membrane potential may exert a profound influence on the invasion of perikarya by spikes generated in the axons. In some TRG neurons, experimental depolarization of the membrane to potentials positive to resting potential results in the inability to support spike generation, presumably due to excessive inactivation of voltage- and time-dependent Na\(^+\)-channels and a shunting action of the increased K\(^+\)-conductance. In other TRG neurons, membrane depolarization may lead to the generation of action potentials within perikarya. This phenomenon possibly is a consequence of the partial inactivation of the 4-AP sensitive current and the persistence of \(I(T)\) at depolarized membrane potentials. This may result in a heightened state of excitability of some TRG neurons which manifests as increased membrane resonance observed in frequency-domain studies (cf. Puil et al., 1987; 1988). The present investigations provide direct evidence that under certain conditions, modulation of sensory impulses may occur prior to the first synaptic junction in the CNS, in the perikarya of sensory neurons.
4.7 Membrane responses to autacoids

The unresponsiveness of TRG neurons to applications of bradykinin was surprising, especially since the majority of TRG neurons in tissue culture have been observed to respond to applications of this peptide (Baccaglini and Hogan, 1983). Moreover, a recent study has provided evidence for the presence of receptors for bradykinin on a subset of small diameter neurons within trigeminal and spinal ganglia of guinea pigs (Steranka et al., 1988). The small sample of TRG neurons tested with bradykinin applications did not exhibit spikes with inflections in the repolarization phase suggesting that these cells were not nociceptive C-neurons. In view of the observations that bradykinin applications specifically affect only C-neurons in the nodose ganglia (Weinreich, 1983), this also may be the case in the TRG where neurons which may belong to this category were infrequently encountered in these investigations. A similar situation was encountered in our previous investigations, in which high doses of 5-hydroxytryptamine failed to produce responses during intracellular recording in TRG neurons (Spigelman, 1986; Puil and Spigelman, 1988). The number of stable recordings from cells with long duration spikes was quite limited, presumably because the small diameter of C-cells makes them much more difficult to investigate using microelectrode techniques.

The results obtained with histamine applications represent the first demonstration of the effects of this autacoid on TRG neurons. The large depolarizations observed in 2 of 9 TRG neurons were similar to the depolarizations evoked by substance P applications. Histamine has been shown to produce depolarizations in ~25% of C-neurons in the rabbit nodose ganglion, but was without effect on the A-neurons (Higashi et al., 1982). It is possible that histamine at relatively high doses is capable of causing the
release of substance P within the TRG, the latter tachykinin in turn producing the observed response. Alternatively, the depolarizations may be evoked directly by histamine acting on distinct receptors. The inability to block the histamine-induced depolarizations with a high dose of cimetidine indicates that these receptors probably are not of the $H_2$-variety. In the rat sympathetic ganglion and the frog neuromuscular junction, histamine may facilitate or inhibit transmitter release by acting presynaptically on the $H_1$- and $H_2$-receptors, respectively (Snow, et al., 1980). A further pharmacological characterization was not attempted because of the unspecific actions of available $H_1$-antagonists. In addition, the low percentage of neurons that responded to histamine made difficult an extensive characterization of such responses as opposed to the large number of TRG neurons that responded to substance P applications.

4.8 Ionic mechanism of substance P action

The synthesis and release of substance P from the central and peripheral processes of sensory neurons have been ascertained in several investigations (cf. Introduction). However, the results described here represent the first demonstration of the profound depolarizing actions of the peptide on the perikaryal membranes of primary sensory neurons in mammals. The responses to substance P in many TRG neurons were subject to desensitization following multiple applications of the peptide. Such desensitization has been observed in the receptor-mediated responses to substance P in the rat acinar cells (McMillian et al., 1987). In addition, several neurons exhibited increases in the amplitude of the response to a second application of the peptide, indicating possible receptor sensitization. The possibility that the
increases in the substance P-responses were due to improvements of the membrane properties is unlikely because the input resistance and membrane potential remained stable throughout the recording periods.

The actions of substance P were observed in the voltage-clamp studies on TRG neurons as a slight increase in the evoked inward currents as well as a small but consistent decrease in the outward currents. Because such outward currents in TRG neurons are mostly a result of an efflux of K$^+$ (Spigelman and Puil, 1987), the possibility arises that substance P may produce the depolarization by blocking K$^+$-current(s). The slight decrease in membrane conductance during a response to substance P (cf. Fig. 26B) supports this suggestion. However, a small reduction in K$^+$-conductance alone is not sufficient to account for the large amplitude of the depolarization, unless the actions of substance P are highly voltage-dependent (cf. Nowak and Macdonald 1982). An increase in membrane conductance for Na$^+$, in combination with the decrease in outward currents could give rise to a significant increase in net inward (steady-state) current resulting in a large depolarization. This interpretation is likely in view of: (1) the observed reduction in substance P-responses of neurons bathed in Na$^+$-deficient media and (2) activation of an inward current by substance P in the presence of K$^+$-channel blockers.

The suggestion that the mechanism(s) for the substance P-induced depolarization in TRG neurons of guinea pigs involves an increase in Na$^+$-conductance and a concomitant decrease in K$^+$-conductance is unlike that proposed for mouse spinal neurons in culture (Nowak and Macdonald 1982), rat dorsal horn neurons (Murase et al., 1986) or preganglionic sympathetic neurons (Dun and Mo, 1988). However, the dependencies of the substance
P-induced effects on both Na\(^+\) and K\(^+\) in TRG neurons are similar to observations in inferior mesenteric ganglia of guinea pigs where substance P is a likely transmitter (Dun and Minota, 1981).

In the present studies, the depolarizations evoked by substance P were not blocked in low-[Ca\(^{2+}\)], Co\(^{2+}\)-containing solutions. Murase et al. (1987) have shown that substance P augments a Ca\(^{2+}\)-sensitive slow inward current in voltage clamped spinal dorsal horn neurons of the rat. In their experiments, the substance P-induced responses also were reduced in conditions of reduced (36 mM) extracellular [Na\(^+\)]. Therefore the possibility exists that the inward current evoked by substance P in the rat spinal cord and in TRG neurons may result from a combined influx of Na\(^+\) and Ca\(^{2+}\). This current would likely be mediated by channels that are distinct from those involved in the generation of the Co\(^{2+}\)-sensitive, Ca\(^{2+}\)-spikes observed during combined applications of 4-AP and TEA because substance P responses were not blocked in low-[Ca\(^{2+}\)], Co\(^{2+}\)-containing media. This possibility is supported by the findings that Na\(^+\) and Ca\(^{2+}\) contribute to the resting conductance of TRG neurons. Substance P may augment these conductances, resulting in membrane depolarization.

In the present investigations, the effects of substance P applications on TRG neurons were greatly reduced or completely abolished in Mg\(^{2+}\)-deficient perfusates. These observations have several interpretations. An influx of Mg\(^{2+}\), may produce the inward current evoked by substance P applications, assuming extracellular [Mg\(^{2+}\)] was high relative to the internal [Mg\(^{2+}\)]. In this mechanism, the Mg\(^{2+}\)-influx would not be mediated by Ca\(^{2+}\)-channels that are susceptible to blockade with Co\(^{2+}\). However, the intracellular [Mg\(^{2+}\)] has not been estimated in mammalian
sensory neurons. Estimates of internal $[\text{Mg}^{2+}]$ have been made in frog skeletal muscle where internal $[\text{Mg}^{2+}]$ was found to be higher than the extracellular $\text{Mg}^{2+}$ content (Alvarez-Leefmans et al., 1986).

Secondly, $\text{Mg}^{2+}$ is well known to be a required cofactor of all enzymes that utilize adenosine triphosphate and other nucleotide triphosphates as substrates (Mudge, 1987). If the substance P effects on TRG neurons are at least in part due to decreased activity of an electrogenic $\text{Na}^{+}/\text{K}^{+}$ pump, the removal of $\text{Mg}^{2+}$ from the extracellular medium could inhibit this effect.

Also, external $\text{Mg}^{2+}$ may serve to modulate the substance P-induced responses by direct interactions with the channels mediating the inward current. The modulation of membrane responsiveness by external $\text{Mg}^{2+}$ acting directly on the channels activated by $\text{N}$-methyl-$\text{D}$-aspartate (NMDA) has been observed in neurons of the CNS (Ascher and Nowak, 1988). However, the increases in the NMDA-evoked inward current in the absence of extracellular $\text{Mg}^{2+}$ in central neurons are in contrast to the observed decreases in the responses to substance P in the absence of external $\text{Mg}^{2+}$ in the TRG. Therefore, it is unlikely that the mechanism by which external $\text{Mg}^{2+}$ modulates the responses to NMDA is applicable directly to the responses evoked by substance P in TRG neurons. However, external $\text{Mg}^{2+}$ may be required for the interaction of substance P with its presumed membrane receptors on TRG neurons, in a manner similar to the $\text{Na}^{+}$-dependent receptor-binding of opioid agonists (Snyder, 1978).

4.8.1 Implications for sensory transmission. The findings in TRG neurons of guinea pigs imply a role for substance P in the transfer of sensory information through craniospinal ganglia. The specialized properties of TRG somatic membranes as revealed by previous (Puil et al.,
1986; 1987a; 1987b; 1988) and these investigations may serve to modulate transmission of afferent impulses along the trigeminal nerve. For example, a release of substance P is inferred from the observations of Katz and Karten (1980) who described the envelopment of sensory neurons by substance P-positive varicose fibers in nodose anglia. Pericellular arborizations in the nodose and trigeminal ganglia of the cat have been extensively described in the classical treatises by Cajal (1909). Such release of the peptide may enhance the excitability of TRG neurons by slowly depolarizing their membranes to potentials where membrane resonance comes into play. The "amplification" of excitability may result in the production of ectopic action potentials which would travel along the trigeminal nerve to the central and/or peripheral terminations. Assuming that release of substance P occurs within the TRG, the depolarizations evoked by such release also may provide a negative feedback system for regulating the synthesis of this peptide. Kesseler et al. (1983) have shown that a reduction in mRNA content and synthesis of substance P occur following veratridine- or K⁺-induced depolarizations of neurons within sensory ganglia.

Secondly, the somatic effects of neuroactive substances in craniospinal neurons of mammals may mimic putative transmitter effects on the membrane receptors of the primary afferent terminals. For example, substance P applications have been shown to modify the excitabilities of terminals of identified primary afferent fibers in the spinal cord (Randić et al., 1982). Furthermore, depolarizations of nerve terminals have been observed following substance P applications in chick sympathetic ganglia (Dryer and Chiappinelli, 1985). Hence, substance P may act directly on the membranes of the central and peripheral terminals by a mechanism similar to that described here for TRG perikarya. Thus, an involvement of substance P in
afferent signal transmission is indicated for at least two sites in this cranial nerve, as well as for the second order neurons of the central nervous system (Andersen et al., 1977, 1978).

4.9 Directions for future research

The investigations in TRG neurons have revealed the presence of two outward currents with different sensitivities for $K^+$-channel blockers. Because the kinetics of these currents were only partially resolved with the single electrode voltage-clamp techniques it would be of interest to examine TRG neurons using two electrode voltage-clamp, in order to obtain the steady-state activation and inactivation kinetics for the two currents.

Another intriguing finding was the appearance of prolonged AHPs that followed spikes evoked during the concomitant applications of 4-AP and TEA. It would be of interest to see whether $K^+$-channel blockers selective for the Ca$^{2+}$-activated $K^+$-currents (e.g., apamin) could block the prolonged AHPs. Such experiments should also be performed without adding 4-AP and TEA to the media in order to estimate the contribution of the Ca$^{2+}$-activated $K^+$-current(s) to the excitabilities of TRG neurons.

The results of the investigations on substance P actions in the TRG raise the possibility that this peptide is released within the ganglion. This hypothesis could be investigated in *in vitro* preparations, using established methodology for substance P detection (Olgart et al., 1977).

Extensive pharmacological investigations of autacoid actions in the TRG are desirable. Tachykinins other than substance P (e.g. α and β neurokinins) should be examined for their effects on trigeminal neurons in order to establish whether more than one tachykinin receptor is capable of mediating the observed depolarizations. A variety of antagonists for substance P
effects have been described, although the specificity of these antagonists has been questioned (cf. Jessell, 1983). It would be of interest to investigate their responses in TRG neurons excited by substance P applications.

The relatively slow onset of substance P-induced depolarizations indicates a possible involvement of intracellular secondary messenger. For example, a role for inositol phosphates has been postulated in the responses of rat acinar cells to substance P (McMillian et al., 1987).

Another important area of investigation is the possible interaction of prostaglandins and endorphins with receptors for substance P. Although prostaglandins are not algogenic, except at very high doses (Horton, 1963; Crunkchorn and Willis, 1971), they can augment nociception. This sensitizing action is antagonized by cyclo-oxygenase inhibitors (Ferreira et al., 1973; Lembeck and Juan, 1974) and is considered to be a mechanism for the analgesic effects of drugs such as aspirin and indomethacin (cf. Flower et al., 1987). Furthermore, prostaglandins have been shown to enhance the responses to substance P released from the trigeminal nerve (Ueda et al., 1985). Therefore, it would be of interest to see whether prostaglandins are capable of sensitizing substance P receptors in the TRG. Unlike prostaglandins, endogenous opioid peptides are very potent analgesics which have been proposed to inhibit the release of excitatory transmitters from terminals of nerves conveying nociceptive information (cf. Duggan and North, 1983). A possible interference with substance P-induced responses by enkephalins should be explored.

The electrophysiological identification of various sensory neuron types (e.g. by conduction velocity) is difficult in in vitro preparations of TRG neurons. In order to estimate the conduction velocity of nerve fibers with reasonable accuracy, the required length of fibers exceeds that of axons in
TRG slices. The identification of neurons therefore can be more easily accomplished in in vivo experiments where stimulation of the axons may be accomplished peripherally in the skin or the tooth pulp. This would allow matching of the perikaryal membrane characteristics with the conduction velocities of their axons. As a result of these and other investigations, it is especially important to determine which subsets of neuronal populations are responsive to substance P. This information may be obtained using receptor binding techniques. For example, Hanley et al. (1980) have been able to examine the detailed kinetics of $[^3H]$-substance P binding to membrane receptors in the rat brain. Such studies should be performed using relatively short periods of incubation with substance P or its agonists in order to avoid receptor desensitization and the resulting underestimation of the number of substance P-receptors (cf. McMillian et al., 1987). Another way of determining if receptors for substance P are located on neurons other than those mediating nociception is to perform binding studies following neonatal pretreatment of animals with capsaicin, thus eliminating the small diameter C-neurons from the ganglionic neuronal population (cf. Jancsó et al., 1977).
5 SUMMARY AND CONCLUSIONS

1. The electrical and pharmacological membrane properties of neurons were studied with current-clamp and single electrode voltage-clamp techniques in *in vitro* slices of trigeminal root ganglia of guinea pigs and in human sympathetic ganglia. The trigeminal neurons had some properties that were similar to, and others which distinguished them from other craniospinal neurons. A much smaller sample of human sympathetic neurons showed properties similar to certain reported characteristics of sympathetic neurons in experimental animals.

2. Human neurons exhibited resting potentials and input resistances mostly in the ranges reported for sympathetic neurons in other mammals. The sensitivities of these neurons to specific ionic channel blockers (e.g. TEA, 4-AP, TTX) are similar to those in other vertebrate neurons. These investigations demonstrate for the first time, that human sympathetic ganglion neurons can be studied successfully in *in vitro* preparations, and hence are valuable for direct relevance to the human condition.

3. Two groups of trigeminal neurons could be distinguished on the basis of a presence or absence of a plateau (hump) on the falling phase of evoked spikes. Differences were observed in the sensitivities of non-humped spikes to TTX, indicating similarities in ionic mechanisms of spike generation in trigeminal and other craniospinal neurons. However, the durations of AHPs in both types of neurons were greater than those observed in other mammalian sensory neurons, suggesting stronger inhibition of the postspike excitabilities in trigeminal neurons.

4. Bath applications of TEA and 4-AP, or Cs⁺ applied internally from the recording electrode, produced an increase in input resistance and a decrease in threshold for spike generation in all neurons. Also,
applications of 4-AP increased subthreshold oscillations of the membrane potential and enhanced the repetitive spike firing evoked by intracellular injections of current pulses, without major effects on the spike AHPs. In contrast, the TEA or Cs\(^+\) applications blocked the oscillations and the AHPs, and did not exaggerate repetitive spike discharges. These investigations suggested that several pharmacologically distinct K\(^+\)-currents contribute to the control of excitability of TRG neurons.

5. During combined applications of 4-AP and TEA, long duration spikes and AHPs were evoked with depolarizing current pulses. These were resistant to blockade with TTX but were abolished in media where CoCl\(_2\) was substituted for CaCl\(_2\). Similar spikes were evoked during prolonged recordings with CsCl- or Cs\(_2\)SO\(_4\) -filled electrodes. However in these conditions spikes were followed by afterdepolarizations, suggesting that ionic currents in addition to K\(^+\) may contribute to the postspike events in TRG neurons.

6. During perfusion with TTX, transient outward currents were elicited at the termination of hyperpolarizing voltage commands from holding potentials near -40 mV. The activation of such currents was rapid (<5ms) and inactivation (\(\tau=19\) ms) was complete at potentials within the activation range. The amplitudes of these currents were reduced in conditions of high extracellular [K\(^+\)] and were only slightly affected by inclusion of Co\(^{2+}\) (2 mM) in low-[Ca\(^{2+}\)] perfusates. The observations that applications of a specific K\(^+\)-channel blocker, TEA, and internal Cs\(^+\) produced dose-dependent reductions in the amplitudes of the transient outward currents, whereas administrations of 4-AP or muscarinic agonists at high doses did not greatly affect these currents, suggest a fundamental distinction from similar
outward currents observed previously in other neurons of vertebrates and invertebrates, and hence this transient outward current in TRG neurons is referred to as $I_{(T)}$.

7. The kinetics of $I_{(T)}$ are indicative of its possible participation in the spike AHPs. Therefore, blockade of $I_{(T)}$ by TEA may interfere with the re-activation of voltage-dependent Na$^+$-channels, leading to an observed decrease in the ability of TRG neurons to discharge spikes repetitively.

8. During combined application of TTX (1 μM) and TEA (10 mM), fast activating, sustained outward currents (>1 s) were evoked by depolarizing commands from holding potentials near -70 mV. These currents were blocked completely by the additional inclusion of 4-AP (5 mM) in the perfusing solution. The TEA-insensitive sustained outward currents presumably have a braking influence on repetitive discharge. Conditions that interfere with these currents, such as blockade of K$^+$-channels by 4-AP application which does not produce a significant blockade of $I_{(T)}$, strongly favour the generation of repetitive spike firing in TRG neurons.

9. The investigations using electrical stimulation of axons revealed that changes in the perikaryal resting potential may result in the inhibition of spike invasion into the perikarya, or facilitate the generation of ectopic spike discharges. Applications of 4-AP facilitated the perikaryal invasion of spikes evoked by axonal stimulation, and also induced the appearance of spontaneous, fast depolarizations that reached spike threshold in the absence of electrical stimulation. These investigations provided evidence that spike generation may occur within the perikarya of sensory neurons, and suggest that such behavior may be operative during normal or pathophysiological conditions.
10. Applications of substance P in micromolar doses produced reversible depolarizations in majority of neurons, whereas other autacoids did not have consistent effects. Increases in the repetitive discharge ability of neurons were evident during such depolarizations. Studies on the ionic mechanism of substance P-action revealed that peptide applications resulted in the activation of inward currents as well as a blockade of outward currents. It also was shown that Na\(^+\) and Mg\(^{2+}\) were involved in the mechanism of substance P-actions.

11. The above findings represent the first demonstration of the profound depolarizing actions of substance P on the perikaryal membranes of sensory neurons in mammals. The excitatory actions of this endogenous peptide also give rise to the possibility of physiological actions of substance P at multiple sites in the trigeminal system.
REFERENCES


HISS, E. and MENSE, S. 1976. Evidence for the existence of different receptor sites for algesic agents at the endings of muscular group IV afferent units. Pflügers Arch. 362: 141-146.


HOLTON, P. 1959. Further observations on substance P in degenerating nerve. J. Physiol. 149: 35P-36P.


LEWIS, T. and GRANT, R. T. 1924. Vascular reactions of the skin to injury. Part II. The liberation of a histamine-like substance in injured skin; the underlying cause of Factitious Urticaria of wheals produced by burning; observations upon the nervous control of certain skin reactions. *Heart* 11: 209-265.


REXED, B. and SOURANDER, P. 1949. The caliber of central and peripheral neurites of spinal ganglion cells and variations in fiber diameter at different levels of dorsal spinal roots. J. Comp. Neurol. 91: 297-306.


PUBLICATIONS

Abstracts:


Articles:


Puil, E., R. M. Miura and I. Spigelman, Consequences of 4-aminopyridine applications to trigeminal root ganglion neurons (submitted).


Spigelman, I. and E. Puil, Excitatory responses of trigeminal neurons to Substance P suggest involvement in sensory transmission. Canadian Journal of Physiology and Pharmacology (accepted for publication).

Spigelman, I. and E. Puil, K$^+$-channel blockade in trigeminal ganglion neurons: effects on the membrane voltage responses and outward currents (submitted).