STUDIES INVOLVING SOMATOSTATIN SYSTEMS IN THE RODENT CENTRAL NERVOUS SYSTEM.

by

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ABSTRACT

Somatostatin is a neuropeptide found throughout the brain. Several studies have established its anatomical distribution as being quite heterogenous with relatively high concentrations appearing in the limbic and striatal systems. Presently, very little is known about the functions of somatostatin systems in the brain and how they interact with other transmitter systems. The following report is a summary of experiments undertaken to assess the functional and chemical interactions of somatostatin with other neurotransmitter systems.

Previous studies have established that the dopaminergic inputs to the basal ganglia are important for locomotor activity and reward. These systems have also been implicated in several mental and neural diseases such as schizophrenia, depression, and Parkinson's disease.

In the first experiment, interactions between dopamine and somatostatin systems were examined using paradigms involving behavioural responses to dopamine agonists. Depletion of somatostatin levels by the drug cysteamine was found to attenuate amphetamine- and apomorphine-mediated motor behaviours but not the reinforcing aspects of amphetamine. The second experiment attempted to further characterize the nature of the dopamine-somatostatin interaction by examining the effects of haloperidol, a dopamine antagonist, on central somatostatin levels. Short term treatment with haloperidol decreased striatal somatostatin

levels. Long term treatment (8 months) with haloperidol failed to alter somatostatin levels in the caudate-putamen.

Since somatostatin levels appear to be normal in Parkinsonian brains, the effects of MPTP poisoning in mice on central somatostatin levels was also studied to examine the accuracy of this animal model of Parkinson's disease and examine the effects of dopaminergic lesions on somatostatin levels. The results of this experiment indicate that MPTP causes a dose dependent increase in nigral somatostatin levels without altering striatal or cortical levels. These results are in partial disagreement with results obtained from both post-mortem Parkinsonian brains and primates given MPTP, thereby questioning the accuracy of this mouse model of Parkinson's disease.

The final experiment examined the effects of the anticonvulsant-antidepressant carbamazepine on central somatostatin levels in the rat. Although the chemical mechanisms responsible for the therapeutic effects of carbamazepine are unknown, previous studies have suggested that its efficacy in the treatment of both manic-depression and epilepsy may be associated with the ability of this drug to reduce the abnormal somatostatin levels observed in these diseases. In this experiment, neither acute, chronic, nor withdrawal from chronic treatment with carbamazepine were found to alter the levels of somatostatin in rats. The lack of effects of carbamazepine on basal

somatostatin levels may indicate somatostatin cells are susceptible to carbamazepine only under pathological situations.

Together, these results are discussed in the context of recent observations of abnormal somatostatin levels in several diseases of the central nervous system and provide some insight into the interactions and functions of somatostatin systems in the normal and abnormal brain.

TABLE OF CONTENTS

LIST OF FIGURES

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INTRODUCTION

Somatostatin in the Basal Ganglia

Somatostatin was first recognized as a physiologically active peptide by Brazeau and his collegues (1973) who isolated this peptide from ovine hypothalami and found it inhibited somatotropin (growth hormone) release; thus somatostatin is often referred to as SRIF (somatotropin release inhibiting factor). Subsequent studies have revealed that somatostatin is present in various endocrine cells, in the peripheral nervous system, and throughout various regions of the central nervous system (Beal et al., 1986a; Brownstein et al., 1975; Finley et al., 1981; Johansson et al., 1984; Kobayashi et al., 1977; Vincent et al., 1985). Within the brain somatostatin is found in high concentration in the hypothalamus and the limbic system, intermediate levels in the basal ganglia and cortex, and low or undetectable amounts in the cerebellum and olfactory bulb.

There appears to be at least two physiologically active somatostatin peptides. In the brain somatostatin is mainly present as the 14 amino acid peptide, but a 28 amino acid peptide has also been shown to exist, which contains somatostatin-14 within its structure. Characterization of the somatostatin gene has revealed that preprosomatostatin is 116 amino acids long and is cleaved to a 92 amino acid prosomatostatin (Epelbaum, 1986). It appears that somatostatin-14 can be cleaved from either the prosomatostatin peptide directly (Gomez et al., 1985), or by a trypsin-like conversion from somatostatin-28 (see review by

Epelbaum, 1986). Studies have suggested these two peptides have different distributions in the brain (Patel et al., 1981) which may be due to different regions having different methods of processing somatostatin-28 to somatostatin-14 (Zingg and Patel, 1983).

Within the rat basal ganglia, somatostatin is found in relatively high concentrations in the nucleus accumbens and caudate-putamen, and low levels in the substantia nigra and ventral tegmental area (Beal et al., 1986a; Brownstein et al., 1975; Kobayashi et al., 1977). Beal and his collegues (1983; 1986a) have also shown that within the caudate-putamen, the levels are quite low in the dorsal-lateral region.

Lesion studies in the rat indicate that much of the striatal (nucleus accumbens + caudate-putamen) somatostatin is present in intrinsic neurons. Early studies suggested the hypothalamus as a source for some striatal somatostatin (Palkovits et al., 1980), however other studies have failed to support this (Beal et al., 1985; Crowley and Terry, 1980). Furthermore, lesions of the afferents to the striatum from the cortex, substantia nigra, thalamus and amygdala do not reduce the striatal levels of somatostatin (Beal et al., 1985). In contrast, striatal kainic acid lesions, which destroy the striatal neurons, reduce the levels of somatostatin (Beal and Martin, 1983).

Immunohistochemical studies have also demonstrated the presence of neurons displaying somatostatin immunoreactivity within the striatum (Finley et al., 1981; Johansson et al., 1984; Vincent et al., 1985). Ultrastructural studies indicate that

these cells are medium-sized aspiny neurons (DiFiglia and Aronin, 1982; Takagi et al., 1983; Vincent and Johansson, 1983; Vincent et al., 1982a). These cells have an indented nucleus, and a rich cytoplasm containing well developed Golgi apparatus and rough endoplasmic reticulum. Furthermore, this particular population of striatal neurons has also been shown to contain an NADPH-diaphorase enzyme activity that allows these cells to be selectively stained (Vincent et al., 1983a). This latter characteristic of these cells allows them to be easily differentiated from the other major striatal interneuron population, the cholinergic cells, which are also aspiny but do not display NADPH-diaphorase activity (Vincent et al., 1983b). Immunohistochemical studies have also revealed that some of the somatostatin containing cells in the striatum also contain neuropeptide Y (Vincent et al., 1982a; 1982b; 1983a). This is supported by radioimmunoassay studies showing that these two peptides have a similar distribution in the striatum (Beal and Martin, 1986a).

Immunohistochemical studies of the synaptic connections of striatal somatostatin cells indicate that these neurons receive most of their synaptic input on dendrites, via both symmetric and asymmetric contacts (DiFiglia and Aronin, 1982; Takagi et al., 1983). Somatostatin-immunoreactive terminals appear to form primarily symmetrical synapses with dendrites and spines of other striatal neurons (DiFiglia and Aronin, 1982; Takagi et al., 1983). At present, the transmitters of the cells with which somatostatin containing interneurons make contact is unknown, but

may include the medium spiny neurons which are believed to be the major output neurons of the striatum.

Somatostatin-dopamine interactions

The two main ascending dopaminergic systems in the rat are the nigrostriatal tract to the caudate-putamen originating in the substantia nigra, and the mesolimbic projection to the nucleus accumbens, olfactory tubercle, and the medial prefrontal cortex arising from the ventral tegmental area.

Although these systems have been extensively studied, the synaptic connections they make are still poorly understood. Dopamine terminals in the striatum have been shown to make both symmetric and asymmetric contacts with a wide variety of cells, forming axo-dendritic, axo-spinous, and axo-somatic contacts (Arluison et al., 1984; Freund et al., 1984; Pickel et al., 1981). The neurochemical identities of these postsynaptic cells are mostly unknown.

There is some evidence supporting the view that the activity of central dopamine neurons may be influenced by somatostatin. Both intraventricular (Garcia-Sevilla et al., 1978) and intrastriatal (Beal and Martin, 1984a; 1986b) infusions of somatostatin increase the turnover of striatal dopamine. Somatostatin also appears to increase the spontaneous (Chesselet and Reisine, 1983; Starr, 1982) and K^+-evoked (Starr, 1982) release of tritiated dopamine from rat striatal slices. Similar results have also been observed *in vivo* in the cat caudate nucleus (Chesselet and Reisine, 1983).

Some of the effects of somatostatin might be mediated through cyclic AMP. Intraventricular administration of somatostatin produces an elevation in striatal cyclic AMP levels (Herchl et al., 1977). More recent studies have indicated that somatostatin can inhibit the stimulation of striatal adenylate cyclase activity produced by dopamine (Chneiweiss et al., 1985; Moser et al., 1986).

The dopamine system also appears to be able to modulate the striatal somatostatin neurons. In this regard, chronic administration of neuroleptics produces a reduction in the levels of somatostatin in the striatum (Beal and Martin, 1984c).

Somatostatin in neural disease

There has been considerable interest in the functions of somatostatin in the periphery and its impications in endocrine disorders. For example somatostatin analogs are being tested for treatment of pancreatic endocrine tumours (Wood et al., 1985), diabetes (Gerich, 1976), and acromegaly (Kingsnorth et al., 1986).

Recently, somatostatin levels in the brain have also been shown to be altered in several neuronal disorders. In Huntington's disease, which is characterized by severe loss of striatal tissue, there appears to be a sparing of somatostatin cells (Dawborn et al., 1985; Ferrante et al., 1985) resulting in striatal somatostatin concentrations being greatly elevated in post mortem brains of Huntington's patients (Aronin et al., 1983; Beal et al., 1984; Nemeroff et al., 1983; Sagar et al., 1984).

In contrast, other disorders such as Parkinson's disease (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al., 1984) and schizophrenia (Nemeroff et al., 1983) appear to have normal striatal somatostatin levels.

Most clinical studies use lumbar punctures to study somatostatin and have provided some interesting results. Included in the list of diseases showing abnormal cerebrospinal fluid (CSF) somatostatin levels are: Parkinson's disease (Cramer et al., 1985), Alzheimer's disease (Gomez et al., 1986a; 1986b; Raskind et al., 1986), Huntington's disease (Cramer et al., 1981), depression (Agren and Lundqvist, 1984; Gerner and Yamada, 1982; Rubinow et al., 1985; Rubinow, 1986), and patients with dystonic syndromes (Cramer et al., 1985; Thal et al., 1985).

Drug regimens have also been shown to alter somatostatin levels. As previously mentioned, neuroleptics reduce striatal somatostatin levels in the rat (Beal and Martin, 1984c). In the clinical setting, neuroleptics have also been shown to alter CSF levels of somatostatin-14 and -28 (Gattez et al., 1986). In addition, altered CSF somatostatin levels have been reported in affectively ill patients on carbamazepine, zimelidine (ZMI), or fluphenazine treatment (Rubinow, 1986). With regard to carbamazepine, this drug has been shown to lower somatostatin levels when the levels are abnormal, in both affectively ill humans (Rubinow et al., 1984; 1985; Rubinow, 1986) and in kindled rats (Higuchi et al., 1986).

The purpose of the following set of experiments was to examine further the possible interactions between dopamine and somatostatin in the basal ganglia. The initial experiments involve a behavioural approach to study the interaction. This was followed by two experiments involving the effects of the drugs haloperidol and MPTP on somatostatin levels. Finally, the effects of the antidepressants carbamazepine and desmethylimipramine (DMI) on the levels of somatostatin were examined to determine if these clinically important drugs have some of their effects by altering somatostatin systems.

EXPERIMENT 1. THE EFFECTS OF CYSTEAMINE ON SOMATOSTATIN IMMUNOREACTIVITY AND ON DOPAMINE MEDIATED BEHAVIOURS.

INTRODUCTION

Very little is presently known about the function of somatostatin in various regions of the brain. Infusions of somatostatin into the ventricles have been shown to alter open field activity (Havlicek et al., 1976; Plotnikoff et al., 1982; Rezek et al., 1976; Vécsei et al., 1983a; 1984), stereotypic behaviours (Rezek et al., 1976), and electrical self-stimulation of hypothalamic sites (Vécsei et al.,1982). Direct infusions of somatostatin into the striatum have also been shown to influence behaviour, with low doses increasing locomotion and producing stereotypic scratching and biting, while higher doses produce motor impairment (Rezek et al., 1977).

Numerous behavioural studies involving the dopamine systems have established some of their functions. Two common behavioural tests which appear to be dependent upon dopamine systems are dopamine agonist-induced stereotypy and locomotor activity. Stereotypic behaviours, such as sniffing, chewing, and gnawing in the rat, appear to be mediated via dopaminergic receptors in the caudate-putamen, while locomotor activity appears to involve the mesolimbic pathway to the nucleus accumbens (Kelly et al., 1975). The mesolimbic dopamine system has also been shown to be involved in reward mechanisms using different paradigms, including: electrical self stimulation (Corbett and Wise, 1980), conditioned

reinforcers (Taylor and Robbins, 1984), and place preference conditioning (Spyraki et al., 1983; see review by Fibiger and Phillips, 1986). Recent studies using the place preference paradigm suggest that the mesolimbic mediated reward and locomotor mechanisms may be differentiated (DiScala et al., 1985; Martin-Iverson et al., 1983; 1985; Mithani et al., 1986; Radke et al., 1987a).

A possible useful pharmacological tool for studying somatostatin systems is the aminothiol cysteamine which appears to temporarily deplete the levels of somatostatin and prolactin for approximately one week (Beal and Martin, 1984b; Brown et al., 1983; Millard et al., 1982; Palkovits et al., 1982; Sagar et al., 1982; Szabo and Reichlin, 1981). Cysteamine does not appear to affect other peptides, including vasopressin, enkephalin, VIP, CCK, LHRH (Palkovits et al., 1982) and neuropeptide Y (Chattha and Beal, 1987) and since prolactin is present in very low concentrations in the brain (Fuxe et al., 1977), cysteamine may be used to study central somatostatin systems.

To understand better the interactions between dopamine and somatostatin in the basal ganglia, the effects of reduction of somatostatin by cysteamine on amphetamine-induced locomotor activity, apomorphine-induced stereotypy, and amphetamine-induced place preference conditioning were examined.

METHODS AND MATERIALS

Subjects

Male Long Evans rats (Charles Rivers, weighing 275-350 g were used and housed 4-5 per cage under a 12 h light cycle (08:00 - 20:00) with ad libitum access to food and water. All rats were left in their home cages for at least 1 week after arrival at the laboratory before experiments were conducted.

Drugs

Cysteamine (β-mercaptoethylamine hydrochloride, Sigma) was dissolved in 0.9% saline (100 mg/ml) and injected subcutaneously (s.c.) (1 ml/kg).

D-amphetamine sulphate (Smith Kline and French) was dissolved in saline (1.5 mg/ml) and injected intraperitoneally (i.p.) (1 ml/kg). Apomorphine hydrochloride (Sigma)(0.5mg/ml) was dissolved in 0.9% saline with ascorbate (0.3 mg/ml) added as an antioxidant. This solution was kept protected from light and on ice during the injection procedures. Rats were injected with 1 ml/kg, s.c..

Procedure

Experiment 1a. Locomotor activity was measured in circular (61 cm) activity cages (BRS/LVE), transected by 6 infrared photocell beams. Photobeam interruptions were recorded and analyzed with a NOVA IV/X minicomputer (Data General) equipped with a Manx interface and software (GC Controls). Rats were injected with

cysteamine or saline (n= 11 per group) 4 hours prior to amphetamine injections. One hour prior to amphetamine injections, rats were placed into the locomotor activity cages, and activity was recorded for 1 hour (habituation). Amphetamine was then administered, and activity was recorded for an additional 3 hours. At the end of locomotor testing the rats were killed by cervical dislocation, the brains were removed, the striata dissected out on ice and assayed for somatostatin immunoreactivity as described later (p. 14).

Experiment 1b. Stereotypy was observed while rats were in steel cages (35 x 40 x 18 cm) with a steel mesh floor, tilted mirror underneath and a clear Plexiglas door on one side. Rats that had been pretreated 11 hours previously with cysteamine (n=19) or saline (n=20) were given 1 hour exposure to the stereotypy cages (habituation). They were then (12 hours after pretreatment) injected with apomorphine, and replaced into the stereotypy cages. An observer blind to the treatment of the rats rated their behaviour during 30 second periods every 10 minutes from 5-65 minutes post-apomorphine injection, using the 7-point rating scale of Kelly et al., (1976). This scale has the following points: 0 = asleep or immobile; 1 = active; 2 = active with bursts of sniffing; 3 = active with continuous sniffing; 4 = continuous sniffing in one location; 5 = sniffing with bursts of licking or gnawing; 6 = continuous licking or gnawing. Rats were killed immediately following the last observation period, their brains were removed, the striata were dissected and the levels of

somatostatin immunoreactivity determined by radioimmunoassay as described later (p. 14).

Experiment 1c. Place preference conditioning and testing was conducted with four shuttle boxes (80 x 25 x 36 cm), each divided into 2 compartments (34 x 25 cm), connected by a tunnel (8 x 8 x 6 cm) which could be closed by guillotine doors. Each of the 2 compartments was distinctive in the colour of the walls (solid brown or black with white stripes) and in the type of floor (grid or mesh). Translucent Plexiglas lids allowed for diffuse illumination of the interior of the compartments. Each box was balanced on a fulcrum; the shifting of a rat's weight tilting the box such that the time spent on each side, and the number of crossings could be recorded with electromechanical equipment.

This experiment consisted of three phases. During Phase 1 (Pretest), rats were placed in one of the two compartments (start side) with the doors open, and the time spent in each compartment and the number of crossings from one compartment to the other recorded over a 15 minute period. This procedure was conducted on two consecutive days.

In Phase 2, rats were confined to the non-start compartment immediately after an injection of amphetamine or vehicle and 12-14 hours after an injection of cysteamine or vehicle. This procedure was conducted for each of 4 days (Day 1, 3, 5 and 7 of Phase 2); each rat was injected and placed within the compartment at the same time each day for 30 minutes. On alternate days (Day 2, 4, 6 and 8), rats were confined to the start compartment immediately after injections of saline; there were no injections

made 12-14 hours prior to these injections. Rats were randomly assigned to one of 4 treatment groups (SALINE + SALINE, SALINE + AMPHETAMINE, CYSTEAMINE + SALINE, CYSTEAMINE + AMPHETAMINE; the first drug in each pair refers to the 12-14 hour pretreatment; the second refers to the treatment immediately before compartment confinement; n = 10 per group).

During Phase 3 (Test), a single 15 minute test of place preference was conducted, as in Phase 1. No injections preceded this test. Immediately after this 15 minute test, the rats were killed, their brains removed, and the striata dissected out and the levels of somatostatin immunoreactivity determined by radioimmunoassay as described later (p. 14).

Statistical Analysis

Locomotor activity was subjected to analysis of variance with 2 factors: 1 between factor (drug treatment) and 1 repeated factor (blocks of 10 min). Individual planned comparisons were made following the method of Winer (1971). Peak, total and median stereotypy scores were analysed separately with one-way analysis of variance. Place preference data was subjected to analysis of variance with 2 between factors (cysteamine X saline and amphetamine X saline) and one within factor (second pretest X test). Planned comparisons were conducted. Biochemical assay results were also subjected to analysis of variance.

Biochemical Assays

Tissue Preparation.

Rats were killed by cervical dislocation, the brains were removed, and the striata, including both the caudate-putamen and NAS (bilateral) were dissected out on ice. Tissue for radioimmunoassay was weighed, boiled for 10 minutes in 2.0 N acetic acid and then sonicated. The samples were then centrifuged at 4°C for 20 min at 10,000 rpm, and lyophilized. Samples were stored at -70°C until assayed.

Radioimmunoassay.

Somatostatin-like immunoreactivity was measured using a radioimmunoassay which compared tissue concentrations of somatostatin-like activity to a standard curve formed by varying concentrations of synthetic somatostatin in assay buffer.

The buffer solution used for the assay contained 24 mM sodium barbital, 3.4 mM sodium acetate, 43.6 mM sodium chloride, 0.25 mM thimerosal, 0.5% bovine serum albumin (BSA) and 500 KIU/ml aprotinin (pH 7.4), and was prepared fresh weekly.

The antibody was a monoclonal (Ab 3) that recognizes both somatostatin 14 and 28 in equimolar ratios. The antibody was generously supplied by Dr J. C. Brown, MRC Regulatory Peptide Group, Department of Physiology, U. B. C.. Antibody solutions were prepared from a stock solution, containing 0.1% sodium azide and 0.1% BSA at a 1:1 dilution and kept frozen at -70°C. The stock solution was further diluted 100 fold in a 0.1% sodium azide/BSA solution and stored at 4°C. On the day of the assay

this was further diluted in cold assay buffer to a final concentration of 1:500,000.

Tyrosine-1-somatostatin (Penisula Labs.) iodinated (^{125}I) via the chloramine T method was generously supplied by Dr. K. Kwok, Department of Physiology, U.B.C.., and stored at -20°C. On the day of an assay, the labelled somatostatin was dissolved in 0.1% trifluoroacetic acid and purified with a sep pak column (C_{18} cartridge, Waters Assoc.) using an acetonitrile step gradient ranging from 20% to 40% acetonitrile in 2% steps of 1.0 ml washes. The purified somatostatin was obtained from the 28% acetonitrile wash. The purified label was diluted in buffer solution to obtain a final concentration of approximately 2500 CPM/ 100 µl.

The synthetic somatostatin-14 (Penisula Labs) was stored at -70°C in 10µg aliquots in a solution containing 140mM lactose, 40µM BSA, 10mM citric acid, 100mM formic acid, and aprotinin (80,000 KIU/liter). On the day of the assay, an aliquot was serially diluted in assay buffer to obtain a range of 0.975 to 500 picograms somatostatin per tube and analyzed in triplicate.

Tissue samples were diluted appropriately in assay buffer to obtain results within the sensitivity range of the standard curve (ED20 = 5.0-10.0 pg; ED80 = 70-100 pg) and analyzed in duplicate.

The final volume of 0.4 ml per tube was incubated for 3 days at 4°C. On the third day, separation of the free and bound labelled somatostatin was performed by adding dextran-T70 (Pharmacia)-coated charcoal (Norit-neutral; Fisher) in 0.5% phosphate buffer with 0.1% hormone free plasma. The tubes were

centrifuged for 30 minutes at 3,000 RPM, and the supernatant, containing the bound somatostatin, was removed. The radioactivity in the charcoal pellet containing the free labelled somatostatin was counted on a gamma counter (Packard, Multi Prias 4) equipped with PC-Data Acquisition and Analysis System (PC-DAAS) to analyze the data.

Results were expressed as picograms of somatostatin per milligram tissue wet weight.

RESULTS

Experiment 1a.

Cysteamine injections given 3 hours prior to locomotor testing significantly reduced locomotor activity during the first 30 min of habituation (Figure 1), as indicated by a significant treatment X time interaction ($F(5,80) = 3.64$, $p < 0.01$), and individual comparisons (first 10 min block: $F(1,16) = 83.15$, $p < 0.001$; second 10 min block: $F(1,16) = 23.11$, $p < 0.001$; third 10 min block: $F(1,16) = 5.21$, $p < 0.05$). The activity of the 2 groups did not differ significantly during the remaining blocks of habituation ($F(1,16) < 1.0$ for all 3 blocks).

Following amphetamine injection, the cysteamine pretreated group exhibited less stimulation of locomotor activity than was seen in the saline treated group (Figure 1), with the main effect of cysteamine treatment significant ($F(1,16) = 7.38$, $p < 0.025$). The interaction between treatment and time (blocks of 10 min) was not significant. Planned comparisons revealed that during the first and the third through to the ninth time blocks the

cysteamine pretreated group differed significantly from the controls ($F(1,16) = 14.76, 10.47, 21.08, 17.94, 15.01, 14.92, 8.25, 7.68$, respectively, $p < 0.025$ in all cases).

Examination of the striatal somatostatin levels for these two groups of rats revealed a significant reduction ($F(1,20) = 6.69$, $p < 0.02$) in the cysteamine group (38.12 ± 1.20 pg/mg) compared with the control group (49.40 ± 2.54 pg/mg) (Figure 2).

These results indicate that 4 hour pretreatment with systemic injections of 100 mg/kg cysteamine, reduced both striatal somatostatin levels and amphetamine induced hyperactivity.

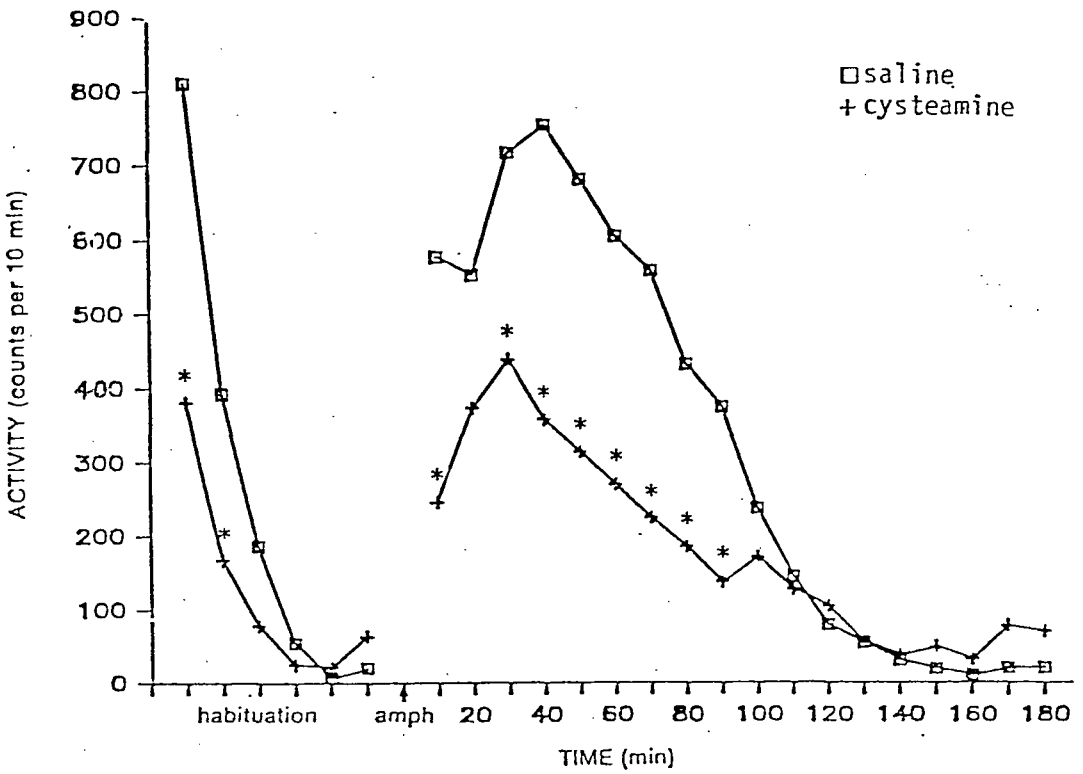

FIGURE 1. Effect of 4 hour pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on amphetamine (1.5 mg/kg, IP) induced locomotor activity. Data represent mean values of beam interuptions per 10 minute block for each group.

* $p < 0.05$ compared to saline group's ten minute block activity.

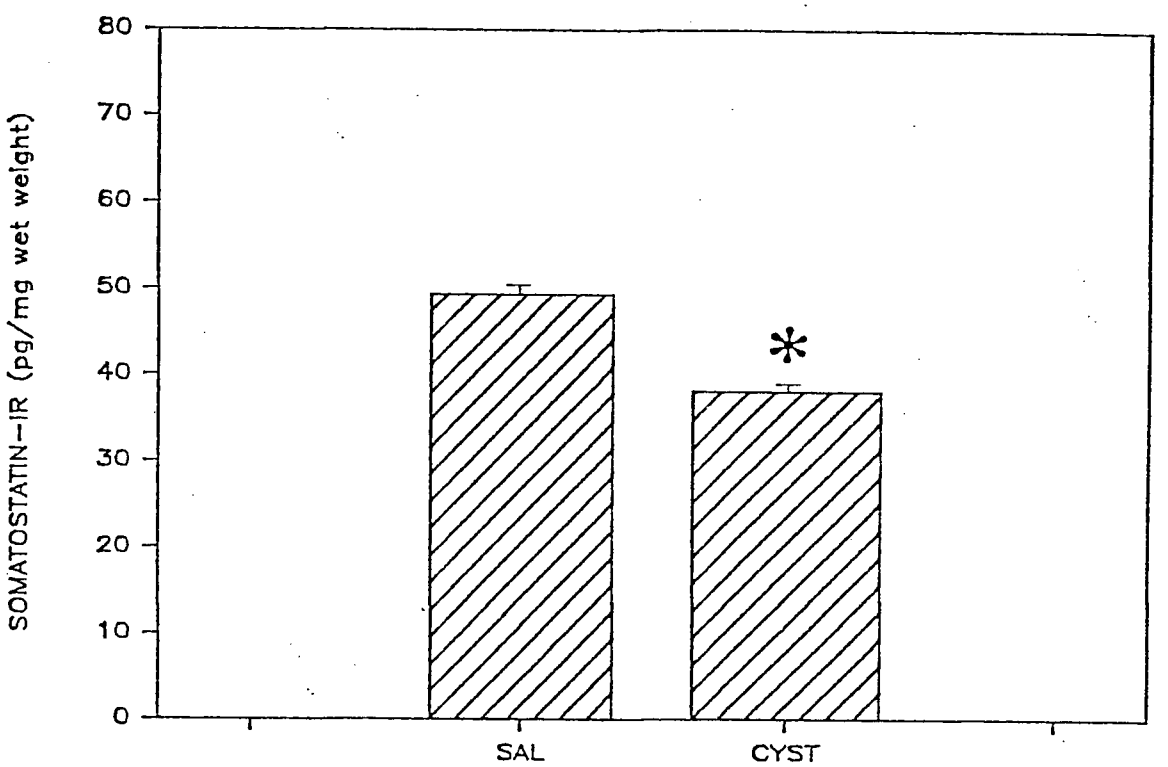

FIGURE 2. Effect of 7 hour pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on striatal (caudate-putamen + nucleus accumbens) somatostatin levels as measured by radioimmunoassay. The tissue obtained in this study were from rats immediately following the locomotor testing. Data represents mean ± SEM values for each group.

* $p < 0.05$ compared to saline group.

Experiment 1b.

As can be observed in Figure 3, the group of rats pretreated with cysteamine 12 hours prior to apomorphine injections exhibited significant reductions in apomorphine-induced stereotypy, relative to the saline pretreated group. This reduction was apparent in both the median ($F(1,37) = 4.25$, $p < 0.05$) and the total ($F(1,37) = 4.81$, $p < 0.05$) stereotypy scores, but apparent reductions in the peak stereotypy scores were not significant ($F(1,37) = 2.13$, $p > 0.1$). Thus, it appears that although apomorphine produced a similar degree (peak effect) of stereotypy in cysteamine pretreated rats as in controls, the stereotypy was of a significantly shorter duration.

The cysteamine treated rats in this experiment had striatal somatostatin levels half that of controls (saline: 59.43 ± 8.61 pg/mg, $n = 9$; cysteamine: 30.38 ± 3.35 pg/mg, $n = 8$; $F(1,15) = 6.28$, $p < 0.025$) (Figure 4). Thus, systemic cysteamine pretreatment significantly depleted striatal somatostatin and decreased apomorphine induced stereotypy.

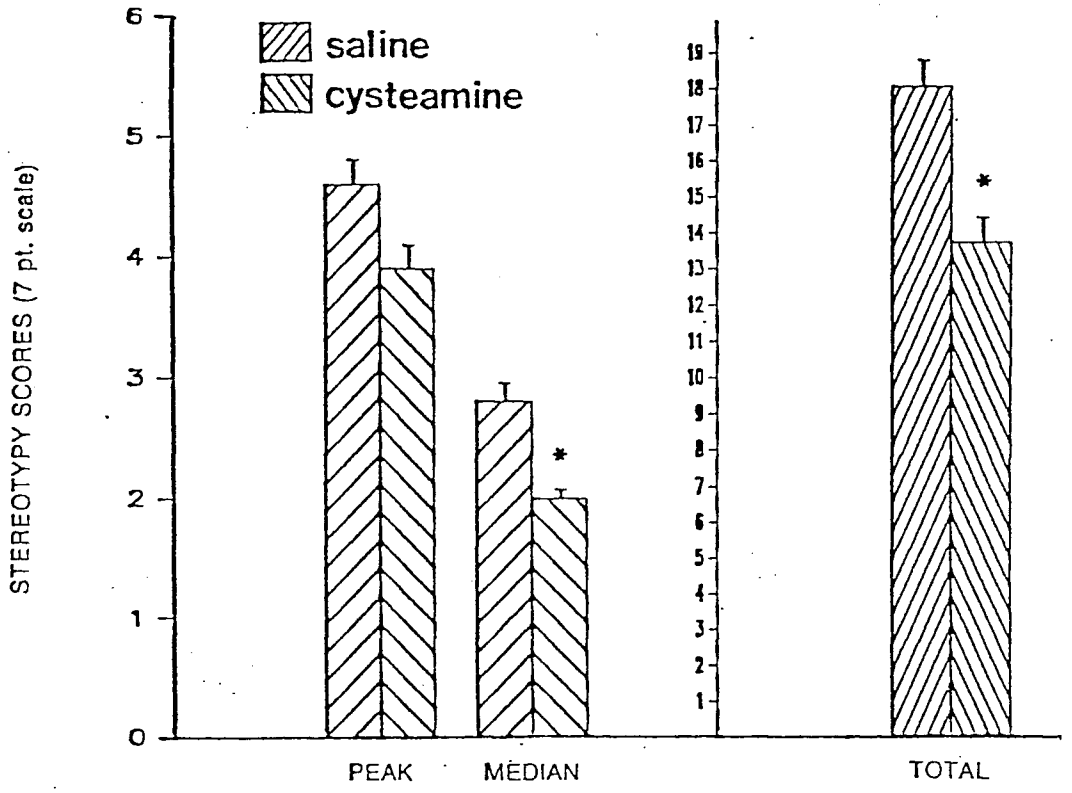

FIGURE 3. Effect of 12 hour pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on apomorphine (0.5 mg/kg, SC) induced stereotypy. Total = sum all 7 scores taken at ten minute intervals, and peak = the highest single score obtained. Data represent mean ± SEM for each group.

* $p < 0.05$ compared to the saline group.

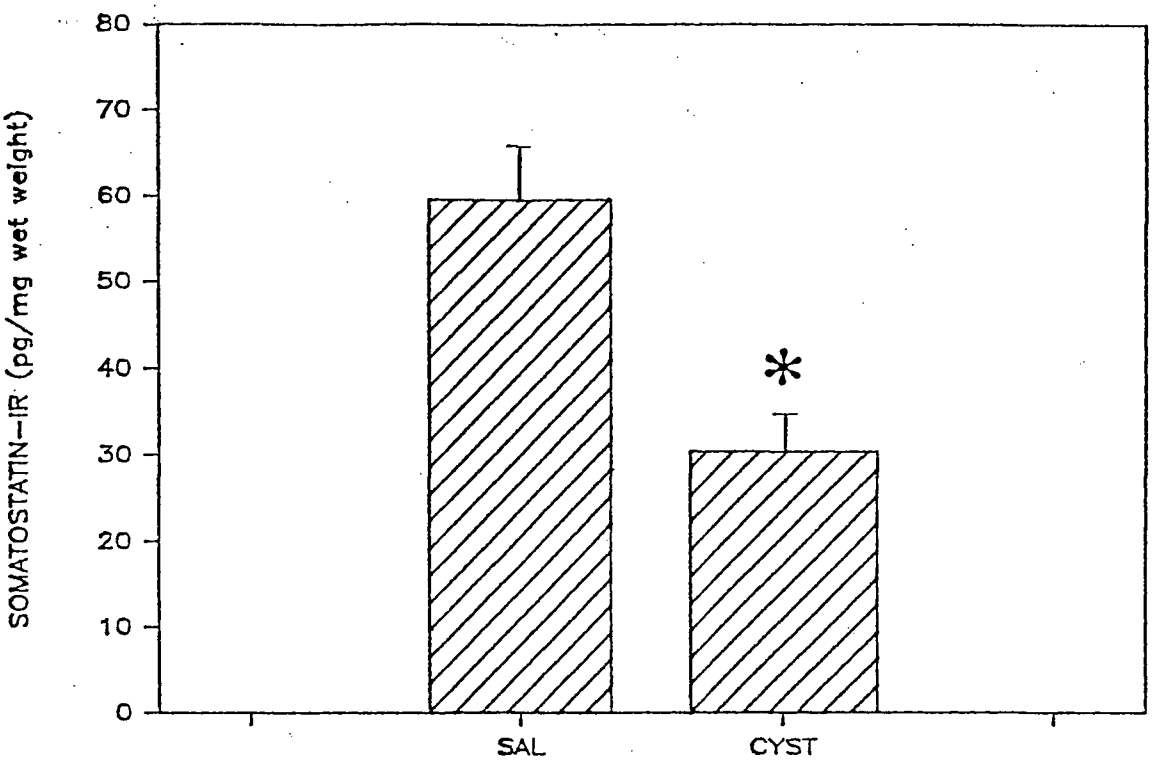

FIGURE 4. Effect of 13 hour pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on striatal (caudate-putamen + nucleus accumbens) somatostatin levels as measured by radioimmunoassay. The tissue obtained in this study were from rats immediately following the stereotypy testing. Data represents mean ± SEM values for each group.

* $p < 0.05$ compared to saline group.

Experiment 1c.

The influence of cysteamine pretreatment on amphetamine-induced place preferences is depicted in Figure 5. Analysis of variance revealed that there was no interaction between cysteamine and amphetamine on the change in place preference from pretest to test ($F(1,30) = 0.62$, $p > 0.1$), but that there was a significant effect of amphetamine, increasing the time spent in the amphetamine-associated compartment on the test day, relative to the last pretest ($F(1,30) = 5.12$, $p < 0.05$). Planned comparisons indicated that while the amphetamine-treated groups exhibited an increase in time spent in the conditioned compartment ($F(1,30) = 5.63$, $p < 0.025$), the saline treated groups did not ($F(1,30) = 0.54$, $p < 0.1$). There was no cysteamine X test interaction evident ($F(1,30) = 0.16$, $p > 0.1$). Thus, repeated cysteamine pretreatment was without significant effect on amphetamine conditioned place preferences.

Cysteamine pretreatment produced a substantial (65.2%) depletion of somatostatin in both saline and amphetamine conditioned groups ($F(1,16) = 34.4$, $p < 0.005$) (Figure 6). Somatostatin levels in saline pre-treated rats were observed to be higher than those from rats in the other experiments; this may be due to the extensive handling the rats received in the place preference experiment relative to the locomotor and stereotypy experiments, or to the influence (possibly stressful) of repeated injections. Amphetamine treatments had no significant effect on somatostatin levels in the vehicle pretreated group ($F(1,16) =$

1.27, $p > 0.1$), nor did it interact significantly with cysteamine treatments ($F(1,16) = 2.58$, $p > 0.1$).

DISCUSSION

The present results indicate that striatal somatostatin depletions produced with cysteamine are associated with an attenuation of the motor effects of dopamine agonists. Vécsei et al. (1984) have previously reported that intraventricular injections of cysteamine 4 hours before behavioural testing reduces spontaneous locomotor activity. In agreement with this, cysteamine given subcutaneously 3 hours before testing reduced locomotor activity during habituation (Figure 1). Amphetamine induced motor stimulation was also attenuated by this treatment 4 hours after cysteamine. That this latter attenuation is a result of a modulation of the actions of central dopamine activity, and not merely a reflection of lower basal activity levels, is supported by a study performed in collaboration with Drs. Martin-Iverson and Vincent. In this study (Martin-Iverson et al., 1986) rats receiving 11 hour, instead of 3 hour, pretreatment with cysteamine (100 mg/kg, s.c.) showed no change in habituation activity but did show a significant reduction in amphetamine-induced locomotor activity. Furthermore, intra-accumbens

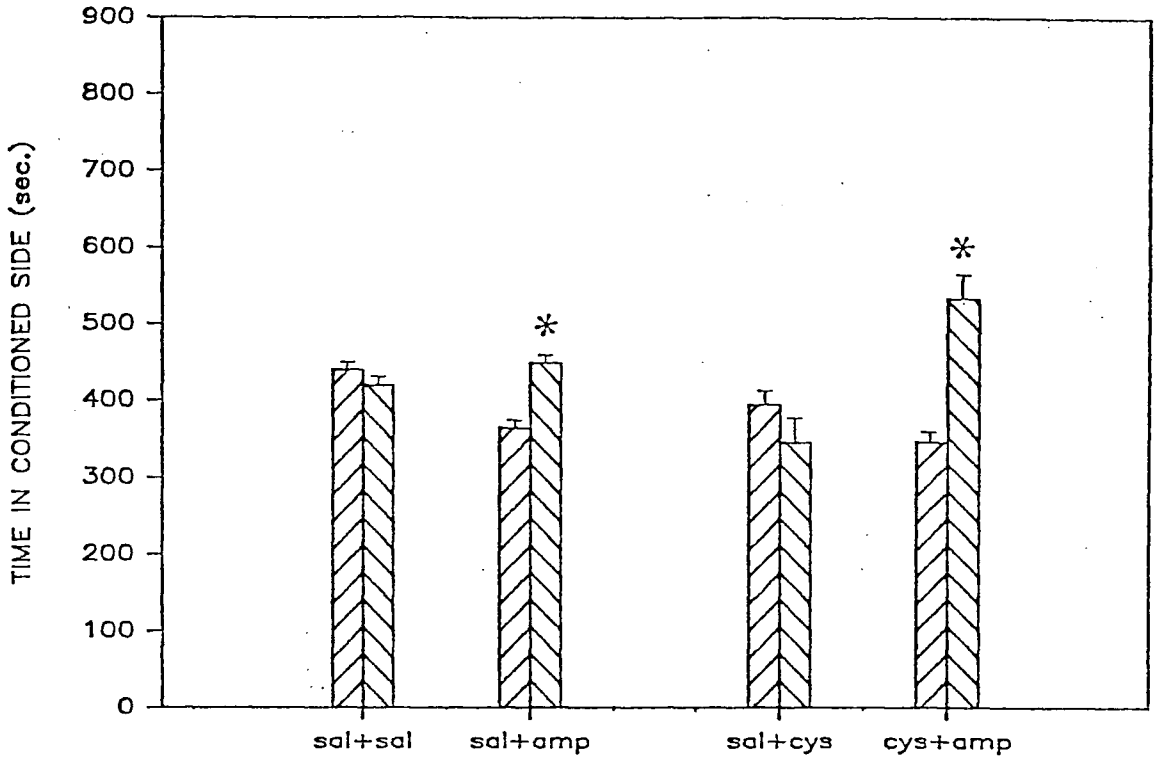

FIGURE 5. Effect of 12 hour pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) during conditioning, on conditioned place preference with saline or amphetamine (1.5 mg/kg, IP). Data represent mean values ± SEM of time spent by each group in a shuttle box compartment before (pretest) and after (test) the conditioning.

* indicates the test times were significantly different compared to the pretest times, $p < 0.05$.

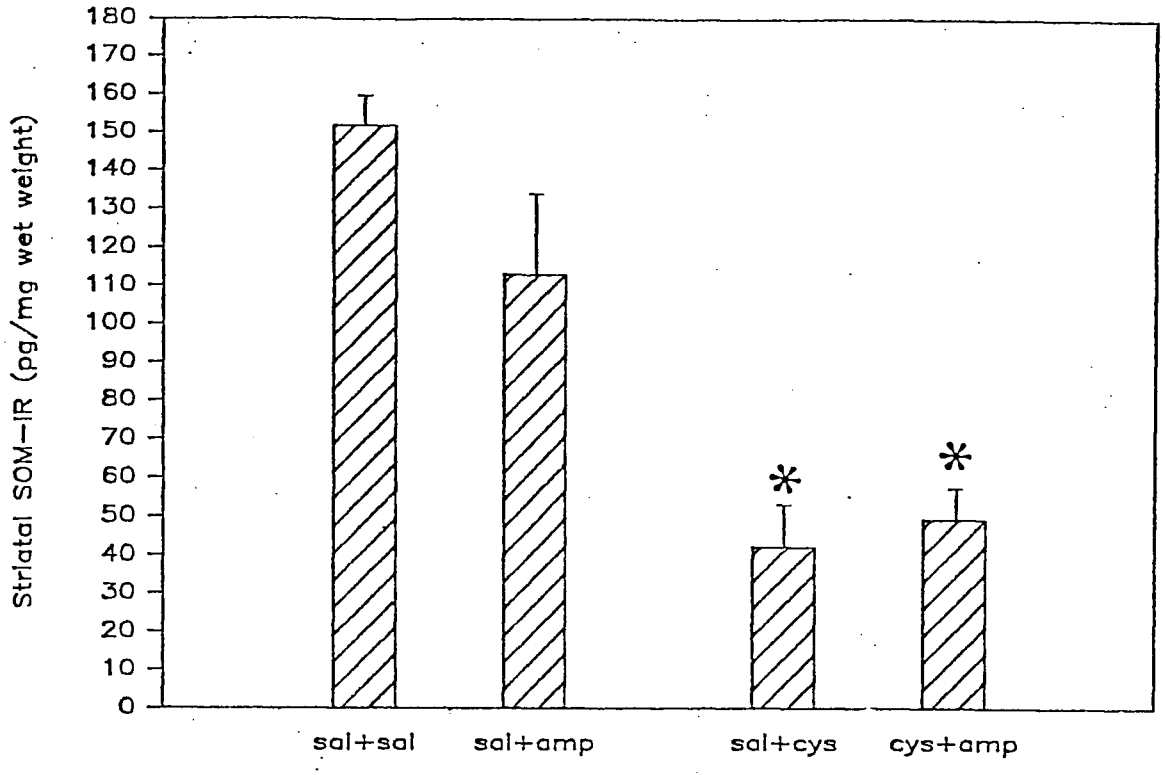

FIGURE 6. Effect of four injections of saline or cysteamine (100 mg/kg, SC) given over eight days followed by 3 days of no injections, on the striatal somatostatin levels as measured by radioimmunoassay. Tissue samples were obtained from rats immediately following the final test session of place preference conditioning. Data represent mean values ± SEM for each group.

* $p < 0.05$ compared to saline groups.

cysteamine injections were also found to attenuate amphetamine-induced locomotor activity without altering habituation activity (Martin-Iverson et al., 1986). These results indicate that somatostatin reductions by either systemic or intra-accumbens cysteamine injections correlate with a reduction in dopamine mediated locomotor activity. The observed reduction in habituation activity (Figure 1) after 3 hour pretreatment but not after 11 hour pretreatment (Martin-Iverson et al.,1986) is similar to the results observed by Vécsei et al. (1984) showing 4 hour, but not 24 hour, pretreatment with cysteamine to reduce open field activity, even though cysteamine reduces somatostatin levels for over 72 hours (Beal and Martin, 1984b; Martin-Iverson et al., 1986). The early change in activity may reflect a stressful factor associated with the initial changes in somatostatin levels.

In addition to cysteamine altering amphetamine induced locomotor activity, peripheral administration of cysteamine, which reduces striatal somatostatin, attenuates the stereotypy produced by apomorphine (Figure 3). As apomorphine is a direct dopamine receptor agonist, this result suggests that somatostatin may modulate the effects of dopamine activity post-synaptic to dopamine-releasing terminals in the striatum. The finding that neither peripheral nor central administration of cysteamine altered dopamine or dopamine metabolite levels (Beal and Martin,1984a; Beal and Martin 1984b; Martin-Iverson et al., 1986) is consistent with this suggestion. In contrast with these results are the observations that somatostatin infusions alter

striatal dopamine turnover (Beal and Martin,1984a; Garcia-Sevilla et.al.,1978) and release(Chesselet and Reisine,1983), and that haloperidol can block the motor effects of somatostatin (Vécsei et al., 1983b). These studies suggests that in addition to somatostatin acting postsynaptic to dopamine terminals, there may also be a feedback mechanism to the dopamine terminals when striatal somatostatin cells are active.

While cysteamine treatment attenuated the motor effects of dopamine agonists, the rewarding action of amphetamine, as revealed by the conditioned place preference procedure, was not reduced. This finding supports previous work suggesting that the motor and reinforcing actions of dopamine agonists are independent of each other (DiScala et.al.,1985; Martin-Iverson et.al.,1985; Mithani et al., 1987; Radke et al., 1987a). For example, a GABA agonist (SL 76002) attenuated locomotor activity, but had no effect on place preference conditioning induced by amphetamine (DiScali et.al.,1985) suggesting GABA and somatostatin cells in the basal ganglia may have opposite actions on the motor stimulant properties of dopaminergic drugs, but neither appears to be essential for the rewarding properties of amphetamine.

While both dopamine-mediated activity (Kelly et al., 1977) and reinforcement (Corbett and Wise, 1984; Fibiger and Phillips, 1986; Spyraki et al., 1983; Taylor and Robbins, 1984) appear to be dependent on the mesolimbic dopamine tract, the observed differentiation of these two behaviours by drug manipulations

indicates further work on this aspect of dopamine function is essential.

It is interesting to note that somatostatin was never depleted more than 65% in the present study even with repeated cysteamine treatments. Previous reports have also noted that depletions of central somatostatin produced by single injections of cysteamine are not complete (Beal and Martin 1984b; Brown et.al.,1975; Palkovits et.al.,1982; Sagar et.al.,1982; Strikant and Patel,1984). This suggests that somatostatin may be differentially compartmentalized; the somatostatin in only certain compartments being susceptible to the actions of cysteamine. This hypothesis is supported by immunohistochemical results (Martin-Iverson et al.,1986) in which cysteamine depleted somatostatin immunoreactivity from nucleus accumbens terminal fields, but not cell bodies. A study by Bakhit et al., (1983) has shown cysteamine to have no effect on somatostatin-28 levels suggesting the somatostatin pool in cell bodies has more somatostatin-28, and the terminals have more somatostatin-14.

Cysteamine appears to be a useful pharmacological agent with which to study the central actions of somatostatin. Prior to this study, cysteamine has been used mostly to study peripheral somatostatin but it has been used to study central somatostatin changes in kindling seizures (Higuchi et.al.,1983; see experiment 3 for detailed discussion).

It is apparent from the present study using cysteamine, that somatostatin may play a role in modulating the motor effects, but not the reinforcing actions, of dopamine agonists. Furthermore,

it is likely that this modulation occurs postsynaptic to the dopamine-releasing terminals in the striatum, since somatostatin depletions reduce the motor effects of both indirect and direct acting dopamine receptor agonists, while not appearing to alter dopamine metabolism. To investigate further the possibility of striatal somatostatin cells being under the influence of dopamine afferents, the effects of chronic haloperidol on somatostatin levels was investigated (experiment 2).

EXPERIMENT 2. THE EFFECTS OF HALOPERIDOL ON SOMATOSTATIN IMMUNOREACTIVITY.

INTRODUCTION

As mentioned in the discussion of experiment 1, there is growing evidence that somatostatin containing neurons in the striatum have their major physiological actions postsynaptic to dopamine terminals. Therefore it was of interest to examine the effects of clinically prescribed drugs, which have been shown to affect dopaminergic systems, on somatostatin levels. One important drug is haloperidol, a direct dopamine antagonist, which is commonly used as an antischizophrenic drug.

Several studies have examined the effects of neuroleptic treatment on non-dopaminergic systems, including those containing GABA (Gunne and HäggstrÖm, 1983), substance P (Hanson et al., 1980), neurotensin (Govoni et al., 1980), cholecystokinin (Frey, 1983), opioids (Hong et al.,, 1978), as well as somatostatin (Beal and Martin, 1984c). These studies are useful for a better understanding of dopamine interactions with other transmitter systems, but they all share a common problem, the length of treatment. In the clinical setting, neuroleptic treatment lasts for several months or years, whereas these studies are generally of a short duration, lasting three or four weeks. Examination of long term treatment in animals would therefore provide a better insight into the neuronal mechanisms involved in maintenance of treatment as well as in the development of the dyskinetic

movements often associated with long term neuroleptic treatment and withdrawal.

The purpose of the following set of experiments was to examine and compare the effects of haloperidol treatment for both three weeks (short term) and eight months (long term) on the levels of somatostatin immunoreactivity in the rat brain. Furthermore, the effect of withdrawal (2 months) of haloperidol after long term administration were also examined.

METHODS AND MATERIALS

Subjects.

Male Wistar rats (Woodlyn) were group housed 4 per cage under a 12 hour light/dark cycle with standard laboratory rat chow provided ad libitum. For the long term studies (8 months), rats began the experiment at a young age, while in the short term experiment, aged rats were used to control for the effects of aging on neurochemistry.

Drugs.

Haloperidol base was dissolved in a 0.5% lactic acid solution (10 mg/ml) and stored at 4°C in the dark. Fresh stock solutions were made monthly. Aliquots of the stock solution were diluted with distilled water and given to the animals as drinking water. Based on a pilot study performed by A. J. MacLennan, the rats consumed 1.3-1.5 mg of haloperidol/kg/day.

Procedure.

This study consisted of three experiments. In experiment 2a (short term treatment), rats were given 3 weeks of haloperidol supplied in their drinking water (1.3-1.5 mg/kg/day) (n=12) or vehicle solution (n=12). In experiment 2b (long term treatment), rats received 8 months of haloperidol (n=10) or vehicle (n=8). In experiment 2c (withdrawal following long term treatment) rats received 8 months of haloperidol (n=8) or vehicle (n=6), followed by 2 months of water. The animals were sacrificed by cervical dislocation between 1:00 and 3:00 pm. Their brains were removed and samples of the nucleus accumbens, caudate-putamen, a cortical sample dorsal to the striatum, medial prefrontal cortex, olfactory tubercle, substantia nigra, and the ventral tegmental area were dissected from sections cut on a freezing microtome, and samples processed for radioimmunoassay as described in experiment 1, except that cold 0.1 N HCl was used instead of boiling samples in 2.0 N acetic acid. Preliminary studies revealed the use of cold 0.1 N HCl did not effect somatostatin immunoreactivity compared to preparing tissue with 2.0 N acetic acid.

Statistics.

Data was expressed as picograms of somatostatin immunoreactivity per milligram tissue wet weight. Mean (± S.E.M) values for each brain region within each group were calculated and analysis between treatment and control groups for each individual brain region was performed using Student's t-test.

RESULTS

As shown in Figure 7, 3 week treatment with haloperidol significantly reduced somatostatin levels in the nucleus accumbens ($t(22)=2.55$; $p < 0.025$) caudate-putamen ($t(22)=3.46$; $p < 0.005$), and the ventral tegmental area ($t(22)=2.34$; $p < 0.05$).

Long term treatment however only reduced somatostatin levels in the nucleus accumbens ($t(16)=2.2$; $p < 0.05$) with levels in all other regions examined not being significantly different from controls (Figure 8). Following withdrawal from long term haloperidol treatment somatostatin levels were not different from control levels in any of the regions examined (Figure 8).

DISCUSSION

The efficacy of neuroleptic treatment appears to be correlated with the antagonistic potency of these drugs on dopamine receptors (Creese et al., 1976). The therapeutic effects of antipsychotics require several days to develop, and animal studies involving neuroleptics have begun to provide some insight into the mechanisms involved in this delay. A popular theory is that continuous haloperidol treatment results in a slow development of depolarization inactivation of dopamine cells (Bunney, 1984; White and Wang, 1983). Although this is a plausible explanation, recent studies have shown that neuroleptics can affect several non-dopaminergic systems

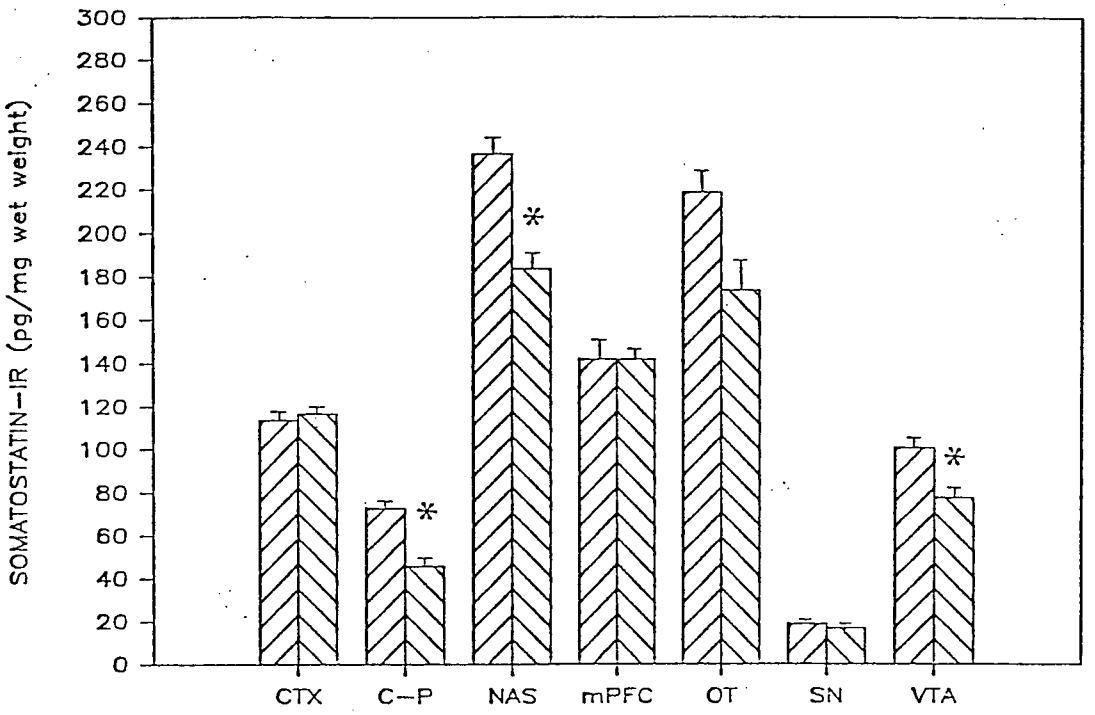

FIGURE 7. Effect of short term (3 weeks) treatment with vehicle (▨) or haloperidol (▧) (1.3-1.5 mg/kg/day) on the levels of central somatostatin immunoreactivity as measured by radioimmunoassay. The brain regions examined include: CTX = cortical sample dorsal to the striatum, C-P = caudate putamen, NAS = nucleus accumbens septi, mPFC = medial prefrontal cortex, OT = olfactory tubercle, SN = substantia nigra, and VTA = ventral tegmental area. Data represents mean values ± SEM for each group.

* $p < 0.05$ compared to control group.

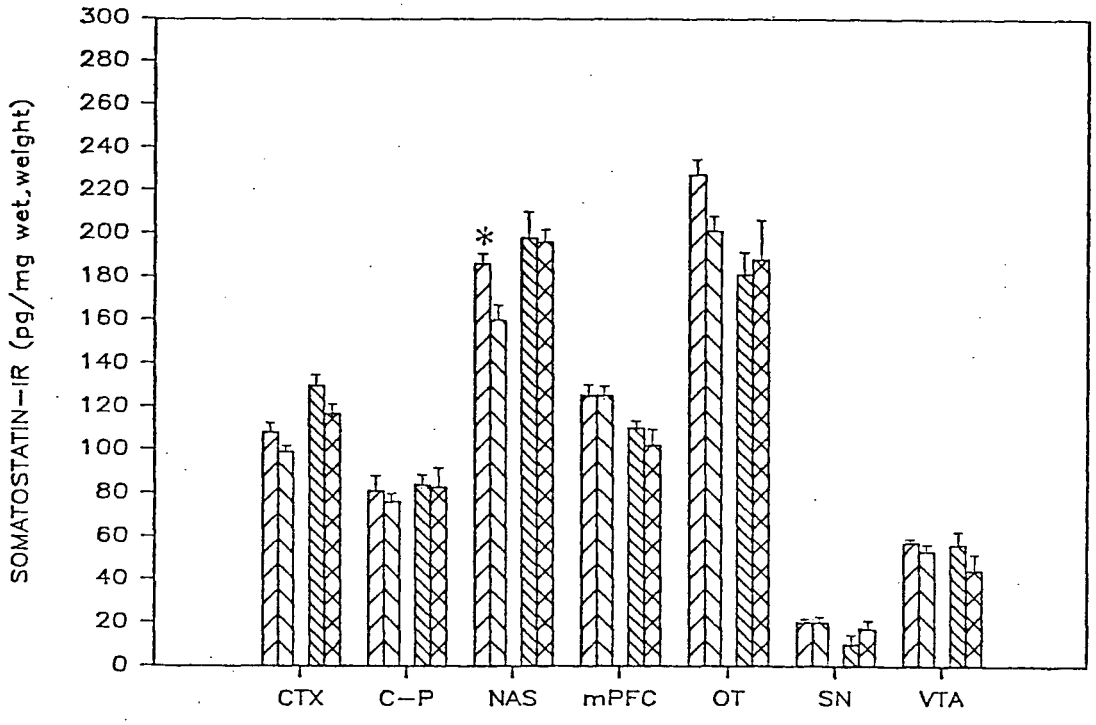

FIGURE 8. Effect of long term (6 months) treatment with vehicle (▨) or haloperidol (▧) (1.3-1.5 mg/kg/day) and the effects of withdrawal from long term treatment with vehicle (▩) or haloperidol (▩) on the levels of central somatostatin immunoreactivity as measured by radioimmunoassay. The brain regions examined include: CTX = cortical sample dorsal to the striatum, C-P = caudate putamen, NAS = nucleus accumbens septi, mPFC = medial prefrontal cortex, OT = olfactory tubercle, SN = substantia nigra, and VTA = ventral tegmental area. Data represents mean values ± SEM for each group.

* $p < 0.05$ compared to appropriate control group.

including those containing GABA (Gunne and HäggstrÖm, 1983), substance P (Hanson et al., 1080; Radke et al., 1987b), opioids (Hong et al., 1978), somatostatin (Beal and Martin, 1984c), cholecystokinin (CCK) (Frey, 1983; Radke et al., 1987c), and neurotensin (Govoni et al., 1980; Radke et al., 1987c). These changes may also be important in the delayed onset of the clinical effects of neuroleptics, and in the side effects of chronic treatment. To provide a more complete understanding of the neurochemical changes occurring during neuroleptic treatment, the effects of various lengths of haloperidol administration on the levels of somatostatin were examined.

The results showed that short term oral treatment of rats with haloperidol significantly reduced somatostatin levels in the nucleus accumbens and caudate-putamen. This agrees with the observations of Beal and Martin (1984c) who gave rats haloperidol injections for 3 weeks and found decreases in the caudate-putamen and the nucleus accumbens. A slight decrease in somatostatin levels in the ventral tegmental area was also found after short term haloperidol treatment. These results support the idea stated in the discussion of experiment 1, that dopamine and somatostatin neurons interact in the basal ganglia. Therefore a decrease in dopamine receptor activation by haloperidol may decrease the activation of somatostatin neurons, which in turn could explain the reduction in striatal somatostatin levels observed. Another possibility would be that somatostatin release is enhanced after haloperidol treatment to compensate for the decreased dopamine activity, thereby reducing the measurable

levels of somatostatin. It should be mentioned that this is very speculative and more detailed examination of the effects of haloperidol on somatostatin synthesis (mRNA levels) or somatostatin release are required to fully understand the mechanisms involved in reducing somatostatin levels.

In contrast to the short term study, long term treatment (8 months) only decreased somatostatin levels in the nucleus accumbens. Somatostatin levels in the caudate-putamen and ventral tegmental area were similar to control levels. These findings suggest that somatostatin containing neurons in the caudate-putamen, but not the nucleus accumbens, adapt over time to the alterations in dopamine receptor blockade. Similar changes have also been observed with neurotensin and substance P (Radke et al., 1987b: 1987c), which are altered after short term treatment but not after long term treatment.

In summary, the effects of short term haloperidol treatment on somatostatin levels in the basal ganglia are consistent with previous studies. In contrast, following long term haloperidol treatment these altered levels return to control values except in the nucleus accumbens where somatostatin levels remained reduced. Furthermore, following withdrawal from long term haloperidol treatment no significant changes in somatostatin levels were detected in any of the brain regions examined. These results provide further evidence for an interaction of dopamine with somatostatin in the basal ganglia, and suggest that somatostatin systems may be important in the clinical side effects associated with long term neuroleptic treatment.

EXPERIMENT 3. THE EFFECTS OF MPTP ON SOMATOSTATIN IMMUNOREACTIVITY IN THE MOUSE.

INTRODUCTION

Lesions of dopamine tracts by 6-hydroxydopamine (6-OHDA) have been shown to alter several transmitter systems, including GABA, acetylcholine (Kim, 1973), cholecystokinin (Chang et al., 1983), and substance P (Hanson et al., 1981). 6-OHDA lesions do not appear to alter striatal somatostatin levels in the rat (Beal and Martin, 1983). This latter finding conflicts with the changes observed in striatal somatostatin levels following short term haloperidol treatment (Beal and Martin, 1984c; experiment 3), but is consistent with studies involving long term neuroleptic treatment (experiment 3), as well as with observations in Parkinson's disease where striatal somatostatin levels are normal (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al., 1984). This suggests that 6-OHDA lesions may be a useful model for Parkinson's disease, with respect to non-dopaminergic aspects of this disease and their implications for treatment.

Recently, the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been shown to cause Parkinson-like symptoms in humans (Langston et al., 1983) and has been used as a model of this disease in both primates (Burns et al., 1983) and mice (Hallman et al., 1984; Heikilla et al., 1983). MPTP has been shown to affect several non-dopaminergic systems, including various neuropeptides (Allen et al., 1986; Zamir et al., 1984).

Since several non-dopaminergic systems are altered in Parkinson's disease (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al., 1984), as well as with 6-OHDA lesions (Chang et al.,1983; Hanson et al.,1981; Kim et al., 1973) and MPTP poisoning (Allen et al., 1986; Zamir et al., 1984), a comparison of the nondopaminergic changes after MPTP and 6-OHDA lesions with those found in Parkinson's disease may establish which of these two drugs produces a more accurate model of Parkinson's disease.

The purpose of the following experiment was to examine the effects of MPTP administration in C57 mice on the levels of somatostatin immunoreactivity to determine if MPTP produces an accurate model of the neurochemical changes observed in Parkinsonism. This study also further examined the interactions between dopamine and somatostatin in the brain.

METHODS AND MATERIALS

Subjects.

Male C57 mice (Charles Rivers) weighing 20-25 grams were group housed (5-6/cage) in polypropylene cages and maintained within a fume hood for the duration of the experiment. Food and water were provided ad libitum.

Drugs.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) (Research Biochemicals Inc.) was dissolved in saline at concentrations of 40 mg/ml, and 50 mg/ml and injected i.p., (1.0ml/100g).

Procedure.

C57 male mice were randomly assigned to one of three groups; a control group (n=7) receiving a daily injection of saline (1.0 ml/100 g) for two days, a second group (n=7) receiving two injections of 40 mg/kg MPTP, and a third (n=6) given two injections of 50 mg/kg MPTP. Fourteen days after the last injection, animals were sacrificed by decapitation and samples of the cortex, caudate-putamen, and the substantia nigra were dissected on ice, and prepared for radioimmunoassay as described in experiment 1. In addition, samples of the caudate-putamen were processed for catecholamine levels using reverse phase high performance liquid chromatography (HPLC) with electrochemical detection (Cumming et al., 1986).

RESULTS

As shown in Figure 9, MPTP poisoning resulted in significant reductions in striatal dopamine levels. For the 2x40 mg/kg group, the reduction was 75 %, and the reduction with the 2x50 mg/kg group was 77 %. There were also decreases in striatal homovanillic acid (HVA), 3,4-dihydroxy-phenyl-acetic acid (DOPAC), and norepinephrine, but no changes were observed in striatal serotonin levels.

Figure 10 illustrates the effects of MPTP poisoning on the somatostatin levels in various brain regions. Somatostatin immunoreactivity in the cortex and striatum was not changed by MPTP poisoning. Instead, there was a significant increase in

somatostatin levels in the substantia nigra that appeared to be dose dependent, with the 2x40 group having a significant increase of 56 % from control values ($t(12) = 3.33$, $p < 0.025$), and the 2x50 group an increase of 72 % ($t(11) = 3.48$, $p < 0.025$).

DISCUSSION

Recent observations of altered peptide levels in post-mortem brain tissues from Alzheimer's disease (Davies et al., 1980), Huntington's chorea (Aronin et al., 1983; Beal et al., 1984; Nemeroff et al., 1983; Sagar et al.,1984) and Parkinson's disease (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al.,1984) have illustrated the importance of neuropeptides in pathology. To develop an accurate animal model of a disease, the neurochemical alterations which may be secondary to the believed major neurochemical dysfunction, should also be consistent with the observed neurochemical changes found in the disease.

In post-mortem studies of Parkinsonian brains no changes in nigral somatostatin levels have been observed (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al.,1984). However, somatostatin levels are reduced in the frontal cortex and hippocampus of demented Parkinsonian patients (Agid and Javoy-Agid, 1985; Epelbaum et al., 1983; Rinne et al.,1984).

In contrast with these findings, our study has shown MPTP poisoning to increase nigral somatostatin levels as the levels of catecholamines decreased. The levels of somatostatin in the cortex and striatum were unchanged.

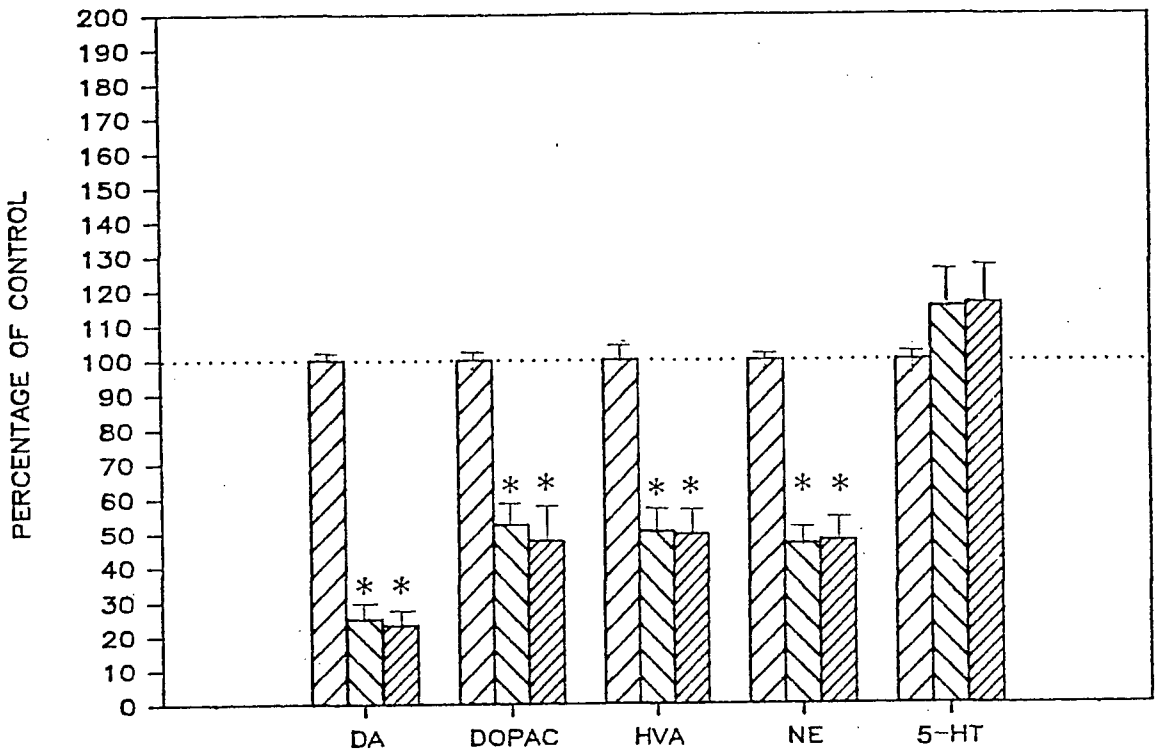

FIGURE 9. Effects of saline (2 x 1 ml/100g) (▨) or MPTP at doses of 2 x 40 mg/kg (▧) and 2 x 50 mg/kg, IP (▨) on striatal catecholamine levels in the mouse. The results are expressed as a precentage of control, with the absolute values of the control groups being, dopamine (DA) = 11,145 ± 324 ng/g wet weight, dihydroxyphenylacetic acid (DOPAC) = 1,323 ± 59 ng/g, homovanillic acid (HVA) = 1,812 ± 137 ng/g, norepinephrine (NE) = 337 ± 69 ng/g, serotonin (5-HT) = 480 ± 15 ng/g.

* $p < 0.05$ compared to control group.

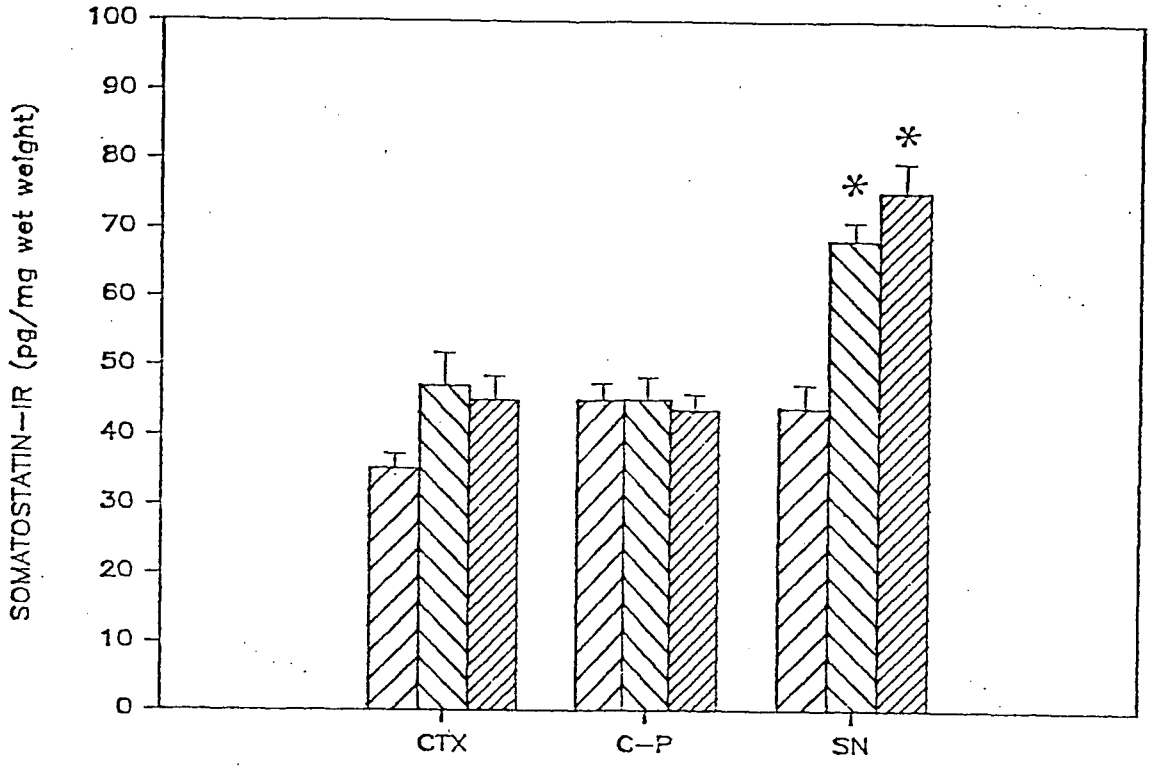

FIGURE 10. Effects of saline (2 x 1 ml/100g) (▨) or MPTP at doses of 2 x 40 mg/kg (▧) and 2 x 50 mg/kg, SC (▨) on the levels of central somatostatin immunoreactivity as measured by radioimmunoassay. Brain regions examined include; CTX = cortical sample dorsal to the striatum, C-P = caudate putamen, SN = substantia nigra. Data represents mean values ± SEM for each group.

* $p < 0.05$ compared to control group.

These results are in partial disagreement with previous MPTP studies in primates. Both Allen et al.(1986) and Zamir et al. (1984), found MPTP to have no effect on nigral somatostatin levels in primates.

The present results with MPTP are similar to earlier studies using 6-OHDA, another proposed animal model for Parkinson's disease. 6-OHDA lesions do not affect striatal somatostatin levels in the rat (Beal and Martin,1983). Beal and Martin (1983) however did not measure nigral somatostatin levels in their study, so it is presently unknown whether nigral somatostatin levels are increased as well, following 6-OHDA lesions.

A possible explanation for the discrepancy between the results of this experiment and studies in other animals given MPTP (Allen et al., 1986; Zamir et al., 1984) is the anatomy of somatostatin systems in the mouse. As shown in Figure 10, the nigral somatostatin levels in the mouse were fairly high, whereas in the primate and rat, the levels in the substantia nigra are quite low (see Figure 7, experiment 2).

The reason why haloperidol does affect striatal somatostatin levels in the rat (experiment 2; Beal and Martin, 1984c) but neither MPTP (Figure 10; Allen et al., 1984; Zamir et al., 1986) nor 6-OHDA lesions (Beal and Martin, 1983) alter striatal somatostatin levels is presently unknown but may indicate that changes in dopamine receptors, but not dopamine levels, have a greater influence on somatostatin neurons.

In summary, mice given MPTP show increases in nigral somatostatin levels. These observations are not consistent with

either MPTP studies in primates, or with findings from post-mortem Parkinson's brains. However, the present investigation does support these earlier studies in showing no reductions in striatal somatostatin levels. The changes observed in nigral somatostatin levels following MPTP may reflect differences in transmitter interactions in different species. Further work involving the interactions of dopamine and somatostatin in different species may help in the interpretation of these results. Finally, these results expand the list of non-catecholaminergic transmitters affected by MPTP. Further work using MPTP should recognize these changes when examining the actions of this toxin.

EXPERIMENT 4. THE EFFECTS OF CARBAMAZEPINE AND DESMETHYLIMIPRAMINE (DMI) ON SOMATOSTATIN IMMUNOREACTIVITY.

INTRODUCTION

As shown in experiments 2 and 3, drugs which disrupt dopaminergic systems appear to alter somatostatin levels. In the clinical setting antidepressants, anticonvulsants (Rubinow et al., 1984; Rubinow, 1986) and neuroleptics (Gattaz et al., 1986), have been shown to alter human CSF somatostatin levels during treatment. In experiment 2, neuroleptics were shown to also affect somatostatin levels in the rat brain, and it is of interest whether somatostatin levels can be altered by other clinically important drugs. One drug of interest is carbamazepine which has been shown to be useful in the treatment of manic-depression, paroxysmal pain disorders, and epilepsy (see reviews by Post et al., 1983 and Rubinow, 1986). Clinically, carbamazepine treatment has been shown to lower CSF somatostatin levels of patients with affective illness, an observation which is fairly unique to this drug since desmethylimipramine (DMI) and lithium do not appear to alter somatostatin levels (Rubinow, 1986). Human studies have also shown a correlation between both carbamazepine treatment and low somatostatin levels with escape from the dexamethasone suppression test (Rubinow, 1986). Whether or not carbamazepine has its clinical efficacy via a direct influence on central somatostatin systems is presently unknown.

The anticonvulsant properties of carbamazepine might also be due to its interactions with central somatostatin systems. Cortical somatostatin levels are increased in animal models of epilepsy (Higuchi et al., 1984; 1986; Kato et al., 1983), and in patients with intractable epilepsy (Nadi et al., 1986). Recently, Higuchi et al. (1986) have shown that carbamazepine decreases somatostatin levels in kindled rats, but only in the brain areas showing seizure-associated elevations in the levels of this peptide. This suggests that carbamazepine might reduce somatostatin levels only if they are abnormal, as in kindled rats (Higuchi et al., 1984; 1986; Kato et al., 1983), or affective illness (Rubinow et al., 1984; Rubinow, 1986).

The purpose of the present study was to determine if acute or chronic administration of carbamazepine alters basal somatostatin levels in the rat. The administration of acute and chronic DMI was also examined as a control group since DMI does not appear to alter CSF somatostatin levels in patients with affective disorders.

METHODS AND MATERIALS

Subjects.

Male Long Evans rats (Charles River) weighing 250-300 grams were group housed 4 per cage under a 12 hour light/dark cycle with food and water provided ad libitum.

Drugs.

Carbamazepine (Sigma) was dissolved in 70% glycerol solution (20 mg/ml) and injected i.p. (1.0 ml/kg). Desmethylimipramine hydrochloride (DMI) (Merrel Dow) was dissolved in distilled water (5.0 mg/ml) and injected i.p. (1.0 ml/kg).

Procedure.

Experiment 4a. Rats were randomly assigned to one of four groups; 1) control group (n=8) given 2 daily injections of vehicle (70% glycerol, i.p.) for 12 consecutive days, 2) acute group (n=7) given 11 days of vehicle injections (2/day) followed by one injection of carbamazepine (20 mg/kg) on day 12, 3) chronic group (n=8) receiving 12 days of carbamazepine (2 x 20 mg/kg), 4) chronic withdrawal group (n=8) receiving 10 days of carbamazepine followed by 2 days of vehicle injections. All rats received their last injection 30 minutes prior to being sacrificed. The hippocampus, hypothalamus, nucleus accumbens, caudate-putamen, and a cortical sample dorsal to the striatum were dissected from sections cut on a freezing microtome and prepared for radioimmunoassay as described in experiment 1.

Experiment 4b. Rats were randomly assigned to one of three groups; 1) control group (n=9) receiving 14 days of two daily injections of vehicle (dH_20, i.p.), 2) acute group (n=9) receiving 12 days of vehicle solution followed by 2 days of DMI (2 x 5.0 mg/mg, i.p.), 3) chronic group (n=7) receiving 14 days of DMI (2 x 5.0 mg/kg, i.p.). All rats received their last injection 12 hours prior to being sacrificed and the nucleus accumbens, caudate-putamen, and a cortex sample dorsal to the

striatum dissected from sections cut on a freezing microtome and prepared for radioimmunoassay, as described in experiment 1.

RESULTS

As shown in Figure 11, neither acute, chronic, nor withdrawal following chronic administration of carbamazepine significantly changed the levels of somatostatin in any of the brain regions examined, when compared to control group mean values. Similarly, acute, as well as chronic DMI administration did not alter somatostatin levels in any brain region examined (Figure 12).

DISCUSSION

The lack of effect of carbamazepine on basal somatostatin levels (Figure 11) would agree with the results of Higuchi et al. (1986) who showed that in regions of the brain with normal somatostatin levels after kindling (ie. hypothalamus, striatum) the somatostatin levels were unaffected by carbamazepine treatment. However, Higuchi et al. (1986) found that the kindling-induced elevation in cortical somatostatin was reduced by carbamazepine using a similar dosage. In the present study, carbamazepine was without effect on basal cortical somatostatin

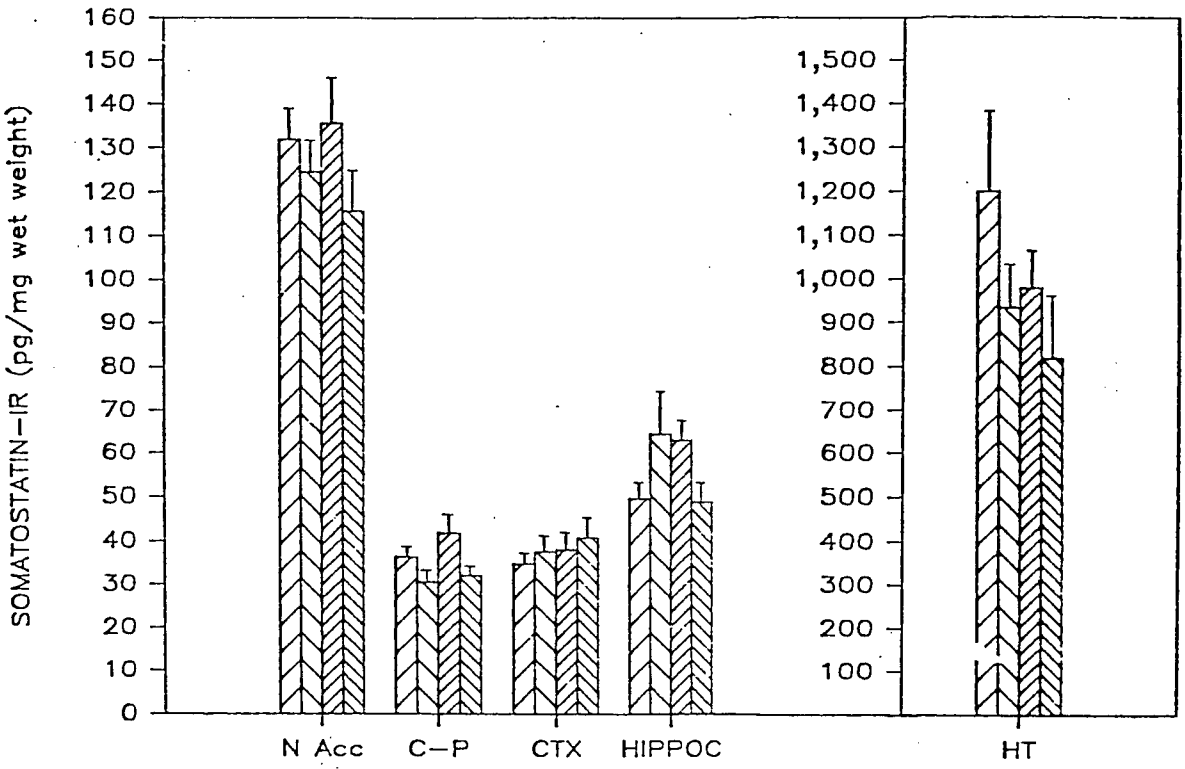

FIGURE 11. Effect of vehicle (▨), acute (▨), chronic (▨), and withdrawal following chronic (▨) treatment with carbamazepine (2 x 25 mg/kg, IP) on the levels of central somatostatin immunoreactivity as measured by radioimmunoassay. Brain regions examined include; N Acc = nucleus accumbens septi, C-P = caudate putamen, CTX = cortical sample dorsal to the striatum, HIPPOC = hippocampus, HT = hypothalamus. Data represents mean values ± SEM of each group.

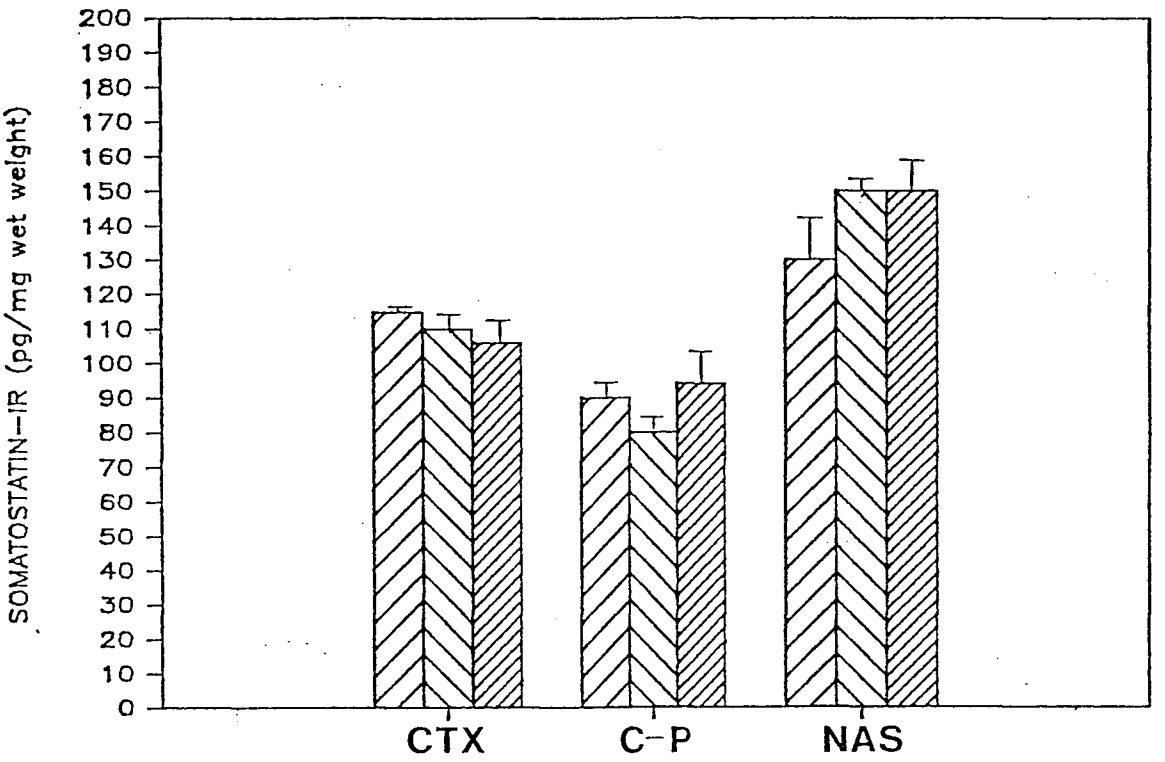

FIGURE 12. Effects of saline (▨), acute (▧), and chronic (▩) DMI treatment on the levels of central somatostatin immunoreactivity as measured by radioimmunoassay. Brain regions examined include; CTX = cortical sample dorsal to the striatum, C-P = caudate putamen, NAS = nucleus accumbens septi. Data represents mean values ± SEM of each group.

levels. Recently, Nagaki et al. (1985) found that carbamazepine at a dose of 50 mg/kg daily (acute and chronic treatment) did not alter central somatostatin levels, however they did observe very slight decreases in somatostatin levels with a very high dose (200 mg/kg) in the cortex (acute) and hippocampus (chronic treatment).

Carbamazepine has been shown to affect central catecholamines (Kowalik et al. 1984; Post et al., 1985; Purdy et al., 1987; Waldmeier et al. 1984), acetylcholine, thyroid hormones, cyclic nucleotides, and GABA (Post et al. 1983). Thus, it is possible that the carbamazepine-induced reductions in somatostatin levels observed by Higuchi et al. (1986) and Rubinow (1986) are the result of carbamazepine's actions on these other systems. A prime candidate might be GABA, since GABA and somatostatin coexist in various regions, including the cortex (Hendry et al. 1984), and cortical levels of GABA are increased by carbamazepine in kindled rats (Higuchi et al. 1986). Therefore it is possible that increases in cortical GABA may be associated with the susceptibility of somatostatin cells to carbamazepine's actions.

With regard to DMI, Rubinow (1986) has shown DMI to have no effect on CSF somatostatin levels in affectively ill patients. In agreement with this, rats given DMI did not show any alterations in central somatostatin levels (Figure 12). A study by Martin-Iverson et al. (1983) has shown DMI to enhance dopamine mediated behaviours, possibly by a non-dopaminergic mechanism. Based on results from experiment 1, showing cysteamine to

attenuate dopamine mediated behaviours, a possible mechanism for DMI to enhance dopamine mediated behaviours would be by increasing striatal somatostatin activity. The results of this experiment indicate DMI is probably not having its effects on dopamine-mediated behaviours via a somatostatin mechanism. Further analysis involving the effects of DMI on other transmitters known to affect dopamine-mediated behaviours, such as acetylcholine (Radke et al., 1987a), GABA (DiScali et al., 1985), substance P (Kelley et al., 1979) or neurotensin (Nemeroff, 1986) may provide some answers to this problem.

In summary, carbamazepine and DMI do not appear to alter basal somatostatin levels in the rat brain. This suggests that carbamazepine may act indirectly to reduce abnormal somatostatin levels. Furthermore, the lack of effect of DMI on somatostatin levels supports clinical observations but fails to establish the mechanisms by which DMI has its effects on dopamine-mediated behaviours. For both drugs, further work to establish the mechanism of action is clearly warranted.

GENERAL DISCUSSION

Dopamine-somatostatin interactions

The effects of somatostatin depletion by cysteamine on dopamine-mediated behaviours (experiment 1; Martin-Iverson et al., 1986) suggests that striatal somatostatin neurons may play a role in modulating the motor, but not the reinforcing actions, of dopamine agonists. Furthermore, it is likely that this modulation occurs postsynaptic to dopamine-releasing terminals in the striatum, since somatostatin depletions by cysteamine reduce the motor effects of both indirect (amphetamine-induced hyperactivity) and direct (apomorphine-induced stereotypy) dopamine agonists. This is further supported by the observations that cysteamine does not appear to alter striatal dopamine metabolism (Beal and Martin, 1984a; 1984b; Martin-Iverson et al., 1986). However, somatostatin infusions into the striatum do alter striatal dopamine turnover (Beal and Martin, 1984a; Garcia-Sevilla et al., 1978) and somatostatin enhances both basal (Chesselet and Reisine, 1983; Starr, 1982) and K^+-induced (Starr, 1982) ^{3}H-dopamine release from rat striatal slices, suggesting that somatostatin can act on dopaminergic terminals. This is further supported by a study by Vécsei et al. (1983b) showing that haloperidol can block the increase in motor activity caused by ventricular somatostatin infusions. Since somatostatin infusions appear to alter dopamine activity, but cysteamine does not, it is possible that somatostatin has both presynaptic and

postsynaptic interactions with dopamine terminals. The presynaptic somatostatin actions may be only effective during increased somatostatin activity since cysteamine does not alter dopamine metabolism, which would be expected if somatostatin had a tonic effect on dopamine terminals.

Based on these observations it would be expected that disruption of striatal dopaminergic activity should affect the activity of striatal somatostatin neurons. This appears to be the case. When animals are treated with haloperidol for 3 weeks (Figure 7), striatal somatostatin levels are reduced. These observations could be interpreted as dopamine receptor blockade causing either a reduction in somatostatin synthesis or an increase in somatostatin release. Future studies involving the effects of dopaminergic drugs on the release and/or synthesis of somatostatin in the striatum may provide a more precise interpretation of these results.

The return of somatostatin levels in the caudate-putamen to control values after long term neuroleptic treatment is interesting in light of the observations of long term neuroleptic treatment being associated with tardive dyskinesia (Casey and Gerlach, 1984). Tardive dyskinesia has been suggested to be due to either dopaminergic supersensitivy and/or cholinergic hypofunction (Casey and Gerlach, 1984), since anticholinergics appear to enhance or stimulate tardive dyskinesia in humans. Another theory stated by Fibiger and Lloyd (1984) is that tardive dyskinesia may be the result of neuroleptic-induced damage to striatal GABA neurons. The proposed synergistic relationship

between dopamine and somatostatin in the striatum is opposite to the believed interaction of dopaminergic neurons with striatal acetylcholine (McGeer et al., 1961; Radke et al., 1987a) or GABA (DiScali et al., 1985) systems. Therefore the observed return of somatostatin to control levels in the caudate-putamen after long term treatment may be related to the development of tardive dyskinesia by increasing the imbalance between neurotransmitter systems, not observed during short term treatment.

Somatostatin in Neuro-psychiatric disease

As mentioned in the introduction, CSF somatostatin levels appear to be abnormal in several neural diseases, including Alzheimer's (Gomez et al., 1986a; 1986b; Rasind et al., 1986), Huntington's (Cramer et al., 1981), and Parkinson's (Cramer et al., 1985) disease, depression (Agren and Lundqvist, 1984; Gerner and Yamada, 1982; Rubinow et al., 1985; Rubinow, 1986), and patients with dystonic syndromes (Cramer et al., 1985; Thal et al., 1985). CSF somatostatin levels are also altered when mentally ill patients are treated with carbamazepine (Rubinow et al., 1984; Rubinow, 1986) or neuroleptics (Gattez et al., 1986).

One problem with measurements of peptide levels in the CSF is the difficulty of interpretation. At present the origins of CSF somatostatin are unclear (Sorenson et al., 1981).

In regard to affective illness, there is growing evidence that somatostatin is an important element in this disease. The reduced CSF somatostatin levels in affectively ill humans has

been correlated with the levels of both noradrenaline (Rubinow et al., 1984) and noradrenaline metabolites (Agren and Lundqvist, 1984). Furthermore, reduced CSF somatostatin levels are correlated with escape from the dexamethasone suppression test (Rubinow, 1986). Treatment with carbamazepine, which has been shown to lower abnormal somatostatin levels in both affectively ill humans (Rubinow et al., 1984; 1986; Rubinow, 1986) and kindled rats (Higuchi et al., 1986) has also been correlated to escape from the dexamethasone suppression test. These observations would suggest that carbamazepine could be reducing hypothalamic somatostatin levels to have its effect on the dexamethasone suppression test since somatostatin has been shown to inhibit ACTH secretion (Brown et al., 1984; Litvin et al., 1986; Reisine, 1985; Richardson et al., 1983).

The results of experiment 4 indicate that carbamazepine does not alter the basal levels of somatostatin in the rat. The term 'basal' is important in discussing these results because carbamazepine appears to reduce somatostatin levels when these levels are abnormal (Higuchi et al., 1986; Rubinow, 1986). This suggests that carbamazepine is not having a direct effect on somatostatin.

Carbamazepine has been shown to affect other neurochemical systems, including GABA, acetylcholine, vasopressin, cyclic nucleotides (see review by Post et al., 1983), substance P (Jones et al., 1985) and catecholamines (Kowalik et al., 1984; Post et al., 1985; Purdy et al., 1977; Waldmeier et al., 1984). Carbamazepine's effects on somatostatin may involve one of these

other transmitter systems since several of them have been shown to affect hypothalamic somatostatin release (Epelbaum et al., 1979; Richardson et al., 1980; Sheppard et al., 1979).

Another possibility, however, would involve a direct effect of carbamazepine on somatostatin neurons during stress. A study by Arancibia et al. (1984) has shown hypothalamic somatostatin release to double when animals are under stress. Since carbamazepine does not appear to alter basal somatostatin levels, its ability to lower abnormal somatostatin (Higuchi et al., 1986; Rubinow et al., 1984; Rubinow, 1986) may involve changes in the susceptibility of somatostatin cells to drugs when under stress, including the stress involved in affective illness or kindling.

Two disorders in which central somatostatin levels are significantly altered, that are pertinent to this report, are Huntington's disease and epilepsy.

In Huntington's disease there appears to be a sparing of striatal somatostatin cells (Dawbarn et al., 1985; Ferrante et al., 1985) and striatal somatostatin levels are elevated (Aronin et al., 1986; Beal et al., 1984a; Nemeroff et al., 1983; Sagar et al., 1984). Recently, experiments in the rat have shown quinolinic acid lesions to spare striatal somatostatin neurons while killing other striatal cells (Beal et al., 1986c), thus providing a useful model of this disease.

Based on the results of the behavioural study presented in this report (experiment 1), the increase in somatostatin levels could contribute to the motor problems seen in Huntington's

disease. Treatment with cysteamine might therefore be useful in re-establishing a balanced neurochemical environment within the diseased striatum. In fact, cysteamine has been recently evaluated as a possible treatment (Shults et al., 1986). Unfortunately, the treatment regimen used failed to relieve the motor abnormalities in this group of patients. This treatment also failed to alter CSF levels of somatostatin suggesting that the dose of cysteamine used was too low. Cysteamine, or similar drugs, might still prove useful in Huntington's patients in future studies.

In intractable epilepsy, cortical somatostatin levels appear to be increased in focal epileptic tissue (Nadi et al., 1986). This is consistent with experiments involving animal models of epilepsy which also show an increase in cortical somatostatin levels (Higuchi et al., 1983; Higuchi et al., 1986; Kato et al., 1983). In the animal models, cysteamine has been shown to decrease both somatostatin levels and seizure activity (Assouline et al., 1984; Higuchi et al., 1983) suggesting that the increased somatostatin levels may be an important factor in epilepsy. Carbamazepine also reduces the increased somatostatin levels and seizures in kindled rats (Higuchi et al., 1986), further suggesting somatostatin is involved in epileptic behaviours. Since both carbamazepine and cysteamine attenuate kindling, but only cysteamine reduces basal somatostatin levels, this suggests that carbamazepine is acting indirectly to decrease somatostatin in kindled animals, but somatostatin is a very important factor in kindling since cysteamine inhibits seizures. Future studies

involving somatostatin in kindling experiments may provide a better understanding of the clinical importance of somatostatin in epilepsy and its treatment.

The functions of somatostatin systems

The functions of somatostatin systems in the brain are obviously different for different brain regions. In the striatum, somatostatin may be important in Huntington's disease and tardive dyskinesia. In the hippocampus and cortex, somatostatin may be a factor in epilepsy. In the hypothalamus, somatostatin may contribute to the hormonal imbalance often occurring in mental illness. Since somatostatin levels appear to be abnormal in several diseases, they may be a useful marker for pathology as first suggested by Patel et al. (1977). But what does somatostatin do in the normal brain? What are somatostatin systems controlling in the striatum, cortex, hypothalamus, etc.? Presently, these questions do not have answers. Future studies measuring somatostatin release in the behaving animal may provide some answers. Until these and other studies have been done, the functions of this interesting peptide will remain obscure.

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APPENDIX 1. Somatostatin radioimmunoassay

The standard curve developed to measure the levels of somostostatin immunoreactivity in tissue was discussed in the methods section of experiment 1 (p.14). For a more complete description of this procedure an illustration of the assay and of a typical standard cureve is shown below.

Protocol for somatostatin RIA

Buffer (µl)	AB (µl)	Tracer (µl)	Standard (100ul)	CPM (mean)	%Bound	SOM-IR (pg/mg)
200	100	100	-	1215	51.4	
300	-	↓	-	2500		
100	100	↓	0.987pg	1277	48.9	
↓	↓	↓	1.975pg	1415	43.4	
↓	↓	↓	3.950pg	1443	42.3	
↓	↓	↓	7.825pg	1532	38.7	
↓	↓	↓	15.62pg	1698	32.1	
↓	↓	↓	31.25pg	1953	21.9	
↓	↓	↓	62.50pg	2266	9.4	
↓	↓	↓	125.0pg	2416	3.4	
↓	↓	↓	250.0pg	2446	2.2	
↓	↓	↓	500.0pg	2442	2.3	
↓	↓	↓	Samples			
↓	↓	↓	#101(0.82mg)	2207	11.7	65.0
↓	↓	↓	#201(0.66mg)	2012	19.5	54.0

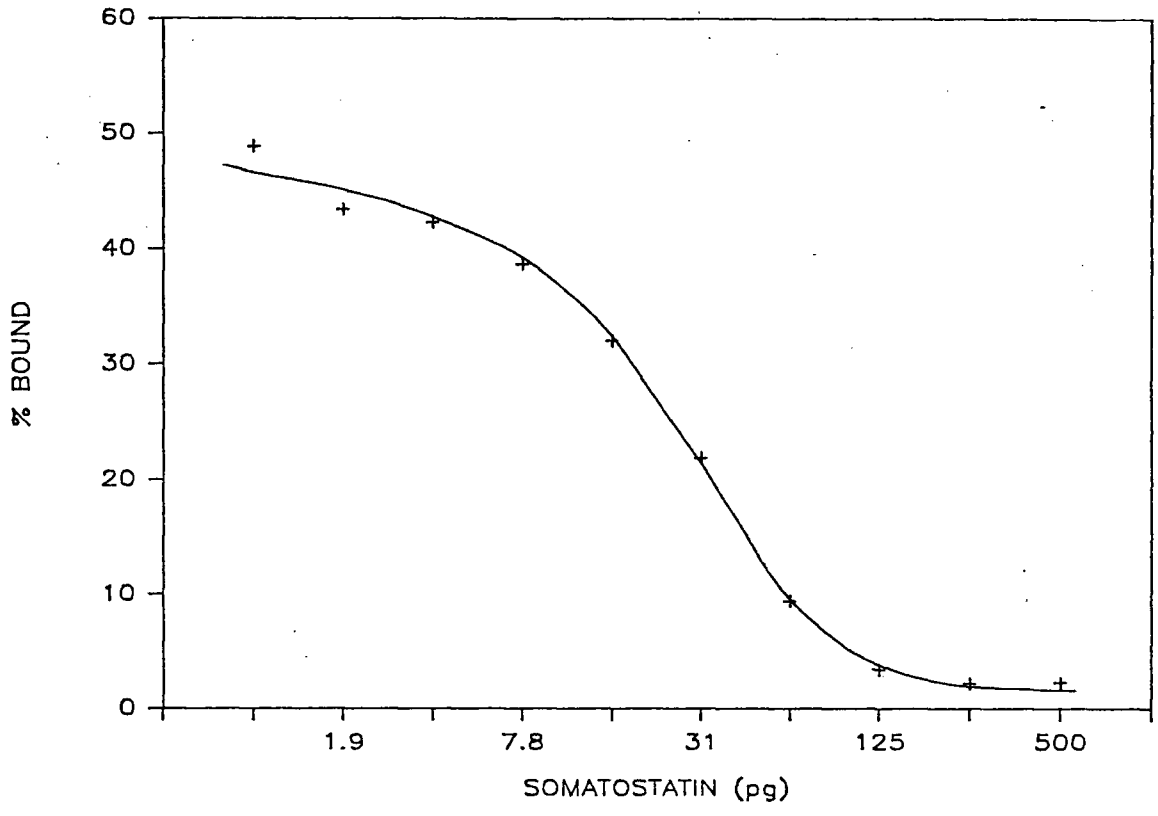

Figure 13. An example of a standard curve used to measure somatostatin immunoreactivity in tissues. The X-axis represents increasing levels of synthetic somatostatin-14 used. The Y-axis is the percent of synthetic somatostatin-14 bound to the antibody which can be calculated using the formula: %B = total counts - standard or sample counts$_x$ 100.