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The Effects of Cysteamine on Dopamine-Mediated Behaviors: Evidence for Dopamine-Somatostatin Interactions in the Striatum

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MARTIN-IVERSON, M. T., J. M. RADKE AND S. R. VINCENT. *The effects of cysteamine on dopamine-mediated behaviors: Evidence for dopamine-somatostatin interactions in the striatum.* PHARMACOL BIOCHEM BEHAV **24**(6) 1707–1714, 1986.—The effects of prior treatment with cysteamine, a drug which appears to deplete selectively the neuropeptide somatostatin, on apomorphine-induced stereotypy and amphetamine-induced locomotor activity and conditioned place preferences were investigated. Twelve hours following systemic cysteamine injections apomorphine-induced stereotypy was attenuated and striatal somatostatin levels were reduced by half. Systemic cysteamine also decreased the motor stimulant effects of amphetamine, without influencing the rewarding properties as determined by the conditioned place preference procedure. Direct injections of cysteamine into the nucleus accumbens also decreased the locomotor response to amphetamine, and produced a local reduction in somatostatin levels in the accumbens. Cysteamine did not appear to alter monoamine turnover in the striatum after either systemic or intra-accumbens injections. These results suggest that somatostatin in the nucleus accumbens and caudate-putamen modulates the motor, but not the reinforcing properties of dopaminergic drugs, possibly via an action postsynaptic to dopamine-releasing terminals. Furthermore, it is evident from these results that cysteamine is an important tool with which to study the central actions of somatostatin.

Somatostatin Cysteamine Dopamine agonists Locomotor activity Stereotypy Place preference

SOMATOSTATIN (SRIF) is a neuropeptide present in high concentrations in the caudate-putamen and nucleus accumbens septi (NAS) [1, 7, 16], where it appears to be contained within medium-sized aspiny interneurons [33]. Little is known of the functional significance of SRIF in these areas. It has been reported that SRIF infused into the ventricular system of rats reduces spontaneous locomotion [6,25], and can induce 'barrel rotation' [9,11]. Other investigators have found that centrally-administered SRIF produces increases, rather than reductions, in spontaneous motor activity [14, 20–22]. Both cyclic and linear forms of SRIF increase ambulation and rearing in a dose-dependent fashion [29]. Dose-related effects appear to account for the discrepancy in reports on the actions of SRIF on locomotor activity: low doses of SRIF infused directly into the neostriatum increase locomotion and produce stereotyped scratching and biting, while higher doses produce motor impairments, interfering with motor coordination [23].

Relatively high doses of SRIF have been reported to depress electrical self-stimulation of hypothalamic sites [31]. However, the doses of SRIF required to attenuate self-stimulation produce significant impairments of motor coordination. Thus SRIF effects on self-stimulation responding may be due to this action, and not to specific effects on the neural substrates underlying reward.

A potentially useful pharmacological tool for investigating the functional role of SRIF is cysteamine, which selectively depletes SRIF-like and prolactin-like immunoreactivities [3, 5, 18, 19, 24, 27, 28]. In the brain, only SRIF appears to be affected directly by cysteamine, and levels of other peptides including vasopressin, enkephalin, VIP, CCK and LHRH are not reduced [19]. Treatment with cysteamine enhances growth hormone secretion [34], lowers body temperature and raises plasma levels of epinephrine, glucose, insulin and glucagon [5]. These effects are opposite to those produced by SRIF, indicating that the decrease in SRIF-like immunoreactivity produced by cysteamine is likely associated with a loss in SRIF biological activity. Cysteamine administered subcutaneously also has behavioral effects opposite of those of centrally administered SRIF, decreasing locomotor

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activity [30]. Recently, direct infusions of cysteamine have been shown to deplete SRIF-like immunoreactivity within the striatum without altering striatal dopamine or serotonin metabolism [3].

There is some evidence supporting the view that the activity of central dopamine (DA) neurons is influenced by SRIF. There have been reports of an increase in striatal DA turnover following intracerebroventricular [13] or intrastriatal [2] infusions of SRIF. SRIF also appears to increase DA release from rat striatal slices and from cat caudate nucleus in vivo [10]. In addition, chronic administration of DA receptor antagonists produces a reduction in SRIF-like immunoreactivity [4].

To understand better the interactions of SRIF and DA in the striatum, we investigated the effects of systemic cysteamine on three behavioral actions of DA agonists that appear dependent upon mesolimbic or nigrostriatal DA projections: apomorphine stimulated stereotypy, d-amphetamine induced locomotor activity [15], and place preferences conditioned with d-amphetamine [26]. Furthermore, the effect of intra-accumbens cysteamine infusions on d-amphetamine stimulated locomotor activity was examined to determine if the effects of systemic cysteamine administration were likely to be peripheral or central in origin.

METHOD

Subjects

Male Long Evans rats (Charles River), weighing 275–350 g were used in all experiments. These animals were housed 4–5 per cage under a 12 hr light cycle (08:00–20:00) and had ad lib 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.

Apparatus

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). Stereotypy was observed while rats were in steel cages (35×40×18 cm) with a steel mesh floor, tilted mirror on the bottom and a clear Plexiglas door on one side. Place preference conditioning and testing was conducted with four shuttle boxes (80×25×36 cm), each divided into 2 compartments (34×25 cm), connected by a tunnel (8×8×6 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.

Drugs

In Experiments 1, 2, 3 and 5, cysteamine (β-mercaptoethylamine hydrochloride, Sigma) was dissolved in 0.9% saline (100 mg/ml) and injected subcutaneously (1 ml/kg). Bilateral intra-accumbens injections of cysteamine were made in Experiment 4. Rats were anesthetized with Halothane, placed in a stereotaxic instrument, and an incision was made along the midline of the head. Holes were drilled into the skull, and a microliter syringe (33 gauge) was lowered to the NAS (coordinates: AP +1.9 mm, ML ±1.5 mm, both from Bregma, and DV −6.4 mm from dura, according to the atlas of König and Klippel, 1963 [26]). Cysteamine (30 μg in 1 μl of 20 mM $NaPO_4$, adjusted to pH 7.1) or vehicle was then injected over a 5 min period. The needle was left in place for an additional 5 min to allow diffusion of the drug. These doses of cysteamine have previously been found to produce a maximal depletion of central [5,24] or striatal [3] SRIF.

In Experiments 2, 3 and 5, d-amphetamine sulphate (Smith Kline and French) was dissolved in saline (1.5 mg/ml) and injected IP (1 ml/kg). For Experiment 1, apomorphine hydrochloride (Sigma) (0.5 mg/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, SC. All drugs were prepared immediately before use.

Procedure

Experiment 1. Rats that had been pretreated 11 hr previously with cysteamine (n=19) or saline (n=20) were given 1 hr exposure to the stereotypy cages (habituation). They were then (12 hr after pretreatment) injected with apomorphine, and replaced into the stereotypy cages. An observer blind to the treatment of the rats rated their behavior during 30 sec periods every 10 min from 5–65 min post-apomorphine injection, using the 7-point rating scale of Kelly *et al.* [15]. 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 SRIF immunoreactivity determined by radioimmunoassay (see below).

Experiment 2. Rats were injected with cysteamine or saline (n=11 per group) 4 hr prior to amphetamine injections. One hr prior to amphetamine injections, rats were placed into the locomotor activity cages, and activity was recorded for 1 hr (habituation). Amphetamine was then administered, and activity was recorded for an additional 3 hr. At the end of locomotor testing the rats were killed by cervical dislocation, the brains were removed, the striata dissected out and assayed for SRIF immunoreactivity as described below.

Experiment 3. Rats were injected with cysteamine or saline (n=5 per group) 12 hr prior to injection of amphetamine. One hr prior to amphetamine injection, rats were placed into the locomotor activity cages, and activity was recorded (habituation). Amphetamine was then injected, and activity was recorded for an additional 3 hr. The rats were then killed, the brains removed, and the striata dissected out and processed for content of dihydroxyphenylacetic acid (DOPAC), DA, 5-hydroxyindole-acetic acid (HIAA), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT) and 3-methoxy-tyramine (3-MT), by high pressure liquid chromatography (HPLC) with electrochemical detection.

Experiment 4. Forty-seven hr after intra-accumbens cysteamine (n=9) or vehicle (n=9) injections, rats were placed in locomotor cages, and activity was recorded for 1 hr (habituation). Amphetamine was then injected, and activity was recorded for an additional 3 hr. After behavioral testing, the rats were killed, their brains were removed, and the

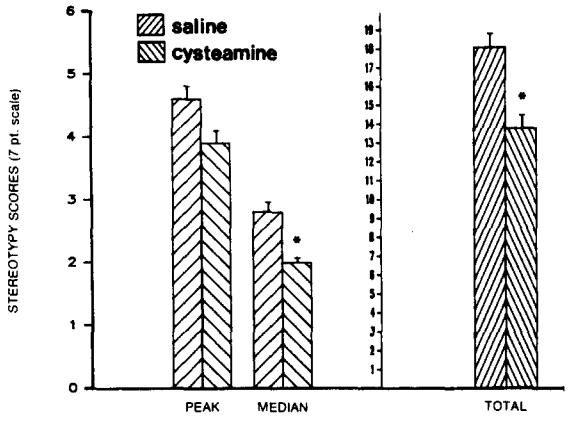

FIG. 1. Effect of 12 hr pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on apomorphine (0.5 mg/kg, SC) induced stereotypy. Total=sum of all 7 scores taken at 10 min intervals, and Peak=the highest single score obtained. Data represent mean± s.e.m. for each group. *$p<0.05$ compared to the saline group.

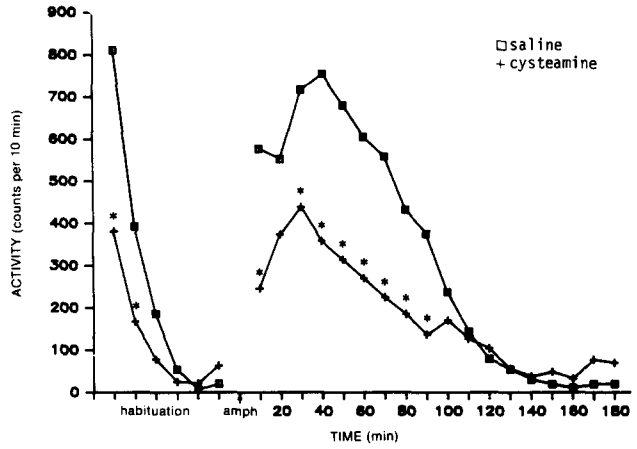

FIG. 2. Effect of 4 hr pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on amphetamine (1.5 mg/kg, IP) induced locomotor activity. Data represent mean values of beam interruptions per 10 min blocks for each group (n=11). *$p<0.025$ compared to the saline group.

NAS, caudate-putamen and a cortical sample dorsal to the striatum were dissected out from sections cut on a freezing microtome and processed for DA, 5-HT, HVA, DOPAC, HIAA and 3-MT content by HPLC.

A similar group of rats was given equivalent intra-accumbens infusions of cysteamine (n=6) or vehicle (n=5), killed 48 hr later, and the NAS, caudate-putamen and a cortical sample dorsal to the striatum were dissected out and assayed for SRIF immunoreactivity.

An additional group of three rats received unilateral intra-accumbens infusions of cysteamine, and were anesthetized with pentobarbital and perfused with a buffered picric acid-paraformaldehyde fixative 48 hr later. Sections from the forebrains of these animals were then stained with cresyl violet or processed for SRIF immunohistochemistry using a monoclonal antibody (Ab 8) as described in detail elsewhere [32]. The SRIF immunoreactivity in the cysteamine treated NAS was examined and compared with that in the uninjected side, and the cannulae placements were confirmed microscopically.

Experiment 5. 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 min 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 hr 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 hr 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 hr pretreatment; the second refers to the treatment immediately before compartment confinement; n=10 per group).

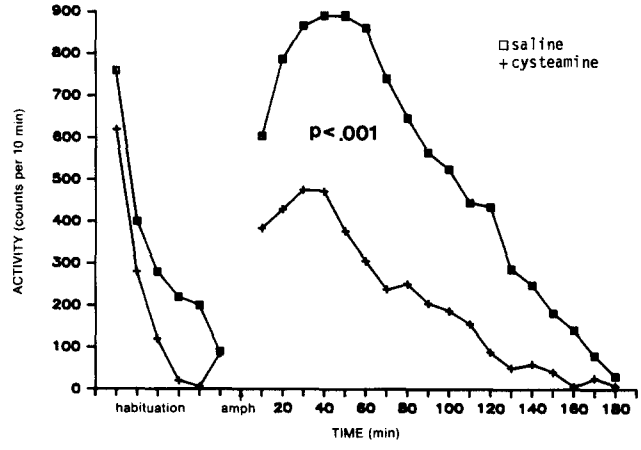

FIG. 3. Effect of 12 hr pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on amphetamine (1.5 mg/kg, IP) induced locomotor activity. Data represent mean values of beam interruptions per 10 min blocks for each group (n=5). Activity during habituation was not affected by cysteamine pretreatment, however, post-amphetamine locomotor activity was significantly attenuated in the cysteamine pretreated group.

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

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 [35].

TABLE 1

EFFECTS OF CYSTEAMINE ON STRIATAL AMINES

Region	Group	DA (μg/mg)	METAB/ DA	5-HT (μg/mg)	METAB/ 5-HT
		A. Experiment 4			
NAS	SAL	13.87 ± 0.50	0.48 ± 0.03	0.72 ± 0.04	1.95 ± 0.07
	CYS	13.20 ± 0.94	0.43 ± 0.02	0.84 ± 0.09	1.89 ± 0.16
Caudate-Putamen	SAL	10.60 ± 0.30	0.33 ± 0.02	0.28 ± 0.02	2.54 ± 0.08
	CYS	10.44 ± 2.2	0.31 ± 0.01	0.25 ± 0.03	2.90 ± 0.33
Cortex	SAL	0.21 ± 0.02	1.15 ± 0.20	0.43 ± 0.02	1.89 ± 0.12
	CYS	0.22 ± 0.03	1.11 ± 0.17	0.44 ± 0.03	2.04 ± 0.22
		B. Experiment 3			
Striatum	SAL	9.69 ± 0.22	0.27 ± 0.01	0.53 ± 0.02	1.35 ± 0.04
	CYS	9.16 ± 0.24	0.21 ± 0.02	0.49 ± 0.02	1.39 ± 0.04

A. Effects of bilateral intra-accumbens injections of saline (1.0 μl) or cysteamine (30 μg/μl) on central amines and metabolites measured 50 hr later. Data represent mean values ± s.e.m. for each group (n=10). METAB/DA represents the molar ratio of DOPAC + HVA + 3-MT to dopamine, and METAB/5-HT represents the molar ratio of 5-hydroxyindole-acetic acid to serotonin (Experiment 4).

B. Effect of systemic injections of saline (1 ml/kg) or cysteamine (100 mg/kg, SC) on striatal amines and metabolites measured 15 hr following injections (Experiment 3).

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 × saline and amphetamine × saline) and one within factor (second pretest × 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 striatum, including both the caudate-putamen and NAS (bilateral) was dissected out on ice (Experiments 1, 2, 3 and 5), or the brains were frozen with CO_2 gas on a freezing microtome, coronal sections were removed, and placed on ice, and the caudate-putamen, NAS and a cortical sample dorsal to the striatum were dissected from each side of the brain (Experiment 4). The tissue for radioimmunoassay was weighed, boiled in 2 N acetic acid and then sonicated. The samples were then centrifuged at 4°C for 20 min at 10,000 rpm, and lyophilized. Samples for HPLC were weighed then sonicated in 2 N perchloric acid containing 0.05% EDTA.

Radioimmunoassay. SRIF immunoreactivity was determined using a monoclonal antibody (Ab 3) [8] and Tyr-1-SRIF labelled with ^{125}I via the chloramine T method. A standard curve was formed with the antibody, labelled SRIF, and synthetic cyclic SRIF (Peninsula Labs., Belmont, CA) in 0.4 ml of 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). Tissue samples were diluted appropriately in assay buffer to obtain results within the sensitivity range of the standard curve (5–500 pg/ml). After a 3 day incubation at 4°C, the free labelled SRIF was separated with dextran-coated charcoal and counted in a gamma counter.

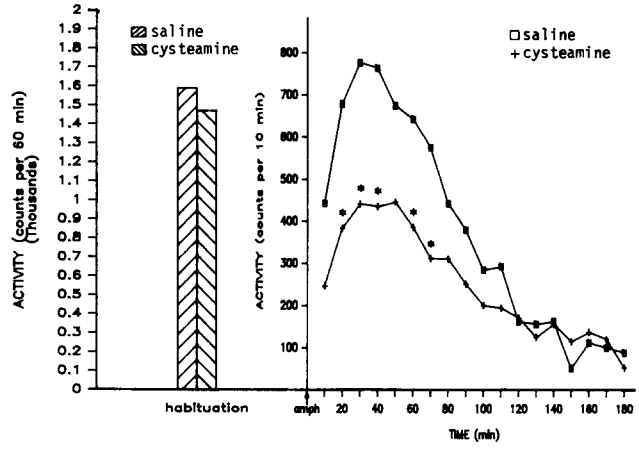

FIG. 4. Effect of bilateral intra-accumbens injections of saline (1.0 μl) or cysteamine (30 μg/μl), 48 hr prior to amphetamine (1.5 mg/kg, IP) induced locomotor activity. Data represent spontaneous locomotor activity [mean values for the total beam interruptions for the habituation period (60 min)], followed by the mean amphetamine-induced activity during each 10 min block following the amphetamine injections. *$p<0.05$ compared to the saline group.

RESULTS

Experiment 1

As can be observed in Fig. 1, the group of rats pretreated with cysteamine 12 hr 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, $F(1,37)=2.86$, $p>0.1$. Thus, it

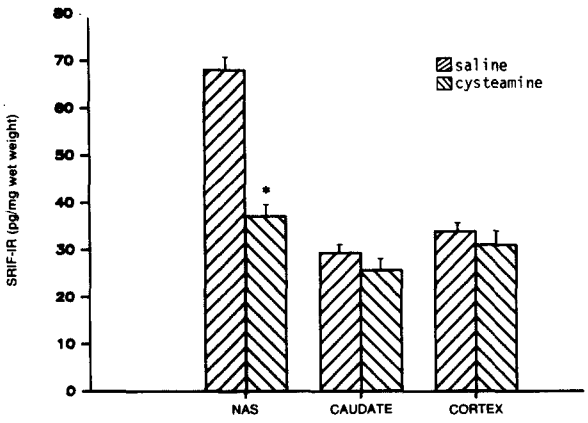

FIG. 5. Effect of bilateral intra-accumbens injections of saline (1.0 μl) or cysteamine (30 μg/μl) on central somatostatin levels. Data represent mean±s.e.m. of somatostatin immunoreactivity (pg/mg wet weight tissue). *$p<0.05$ compared to the saline group.

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.

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

Experiment 2

Cysteamine injections given 3 hr prior to locomotor testing significantly reduced locomotor activity during the first 30 min of habituation (Fig. 2), as indicated by a significant treatment × 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 (Fig. 2), 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 SRIF 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).

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

Experiment 3

Rats pretreated with cysteamine for 11 hr did not exhibit a significant difference from the saline-pretreated group in overall locomotor activity during the habituation period, $F(1,8)=3.93$, $p>0.05$ (Fig. 3). Although the decrease in locomotor activity from the first to the last 10 min period during habituation was significant for both cysteamine and vehicle-pretreated rats, $F(5,40)=39.31$, $p<0.001$, there was no significant interaction between drug pretreatment and blocks of 10 min, $F(5,40)=1.18$, $p>0.05$.

In contrast to the results from the habituation period, post-amphetamine locomotor activity was affected by cysteamine pretreatment 12 hr before amphetamine injections (Fig. 3). The overall locomotor activity measure was significantly lower in the cysteamine-pretreated rats, as compared to the controls, $F(1,8)=25.76$, $p<0.001$. There was also a significant attenuation of locomotor activity through the period of testing, exhibited by both groups of rats, $F(17,136)=27.77$, $p<0.001$, but the treatment × time interaction was not significant. Thus, parenteral cysteamine injections given 12 hr prior to amphetamine, significantly attenuated amphetamine-induced locomotor activity, without influencing spontaneous locomotor activity (habituation).

Assay of levels of monoamines and monoamine metabolites in the striatum with HPLC revealed that this cysteamine pretreatment did not produce a significant effect on any of the neurochemicals measured (Table 1). Furthermore, DA turnover, as assessed by the DA metabolite:DA ratio, was not altered in these animals.

Experiment 4

No significant effect of bilateral intra-accumbens injections of cysteamine 47 hr prior to habituation testing was observed on spontaneous locomotor activity, $F(1,16)=0.09$, $p>0.1$ (Fig. 4). However, there was a significant effect of cysteamine pretreatment on the interaction of post-amphetamine locomotor activity and time, $F(17,272)=2.86$, $p<0.01$. Individual comparisons revealed that the cysteamine-treated group exhibited significantly lower post-amphetamine locomotion from the second through to the seventh 10 min block, $F(1,16)=8.18$, 10.63, 10.18, 4.95, 6.23, 6.55, respectively, $p<0.05$ in all cases, but not during any of the other blocks of time (Fig. 4).

SRIF levels in the NAS of rats given intra-accumbens infusions of cysteamine 48 hr before sacrifice were reduced to half those in the group receiving vehicle injections (Fig. 5). The levels of SRIF in the caudate-putamen and cortex of the cysteamine-treated rats were not significantly different from those in the vehicle group.

Immunohistochemical examination of cysteamine-injected NAS confirmed the results of the radioimmunoassay. The dense SRIF-immunoreactive terminal field present in the normal NAS was absent in the side receiving the cysteamine injection (Fig. 6). However, SRIF-positive cell bodies were present in the injected NAS in the same numbers as seen on the control side. No evidence of non-specific tissue damage, or loss of NAS cells was seen in the cresyl violet sections.

Assay of the NAS, caudate-putamen and cortex for content of DA, 5-HT and their metabolites by HPLC indicated that intracerebral cysteamine had no significant effect on any of these measures (Table 1). Nor was there evidence of a change in DA turnover, as assessed by the DA metabolite:DA ratio, in any of the brain regions examined.

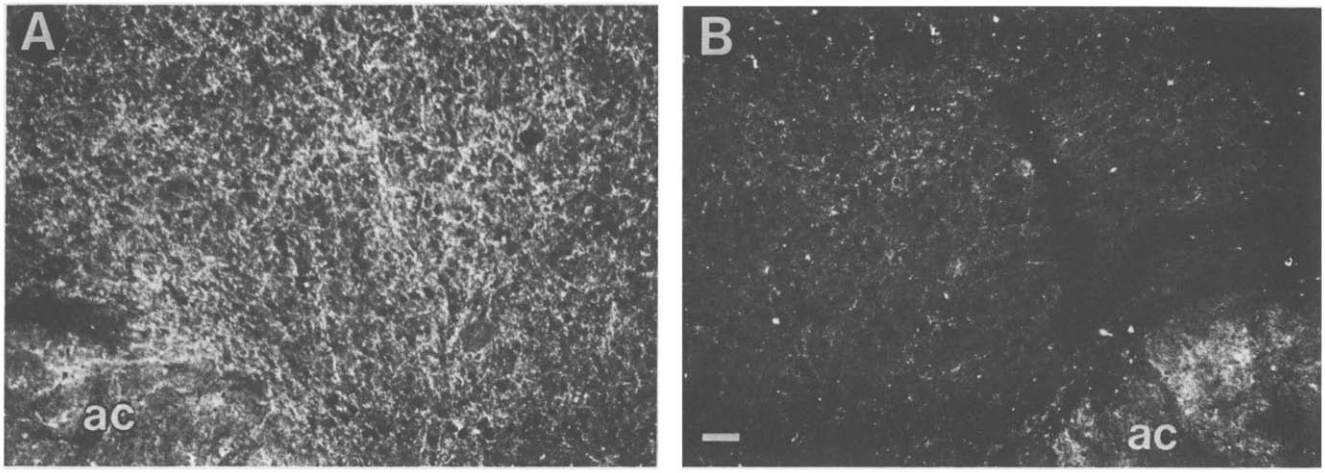

FIG. 6. Immunohistochemical localization of somatostatin immunoreactivity in the normal (A) and cysteamine (30 μg/μl) injected (B) nucleus accumbens. Note that the dense terminal field seen with darkfield optics in the control side is absent from the injected side. ac=anterior commissure, calibration bar indicates 100 μm for both figures.

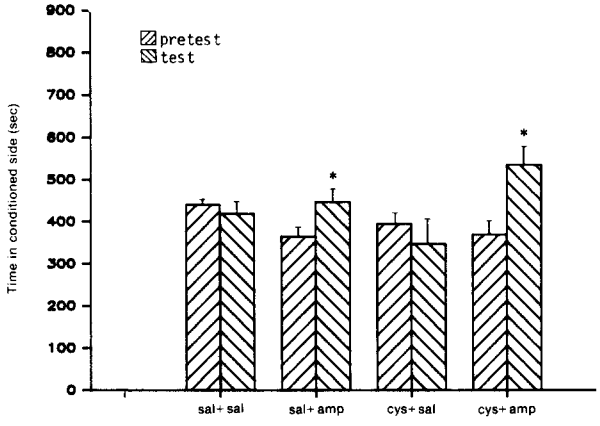

FIG. 7. Effect of 12 hr pretreatment with saline (1 ml/kg) or cysteamine (100 mg/kg, SC) during conditioning, on conditioned place preference with saline or amphetamine (1.5 mg/kg, IP). Data represent mean values±s.e.m. of time spent by each group in a shuttle box compartment before (pretest) and after (test) the conditioning. *Indicates that the test times of the amphetamine groups are different than the pretest times, $p<0.05$.

Experiment 5

The influence of cysteamine pretreatment on amphetamine-induced place preferences is depicted in Fig. 7. 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 × test interaction evident, $F(1,30)=0.16$, $p>0.1$. Thus, repeated cysteamine pretreatment was without significant effect on amphetamine conditioned place preferences.

TABLE 2

STRIATAL SOMATOSTATIN LEVELS IN PLACE PREFERENCE GROUPS

Group	Striatal SRIF-IR (pg/mg wet wt.)
saline + saline	152.1 ± 11.7
saline + amph.	112.0 ± 21.7
cyst. + saline	42.3 ± 12.4*
cyst. + amph.	49.5 ± 10.1*

Somatostatin levels in pg/mg wet weight are given for the four groups used in the place preference study, Experiment 5. The first drug in each pair refers to the 12–14 hr pretreatment, and the second to the treatment immediately before compartment confinement, n=10 per group. *Indicates $p<0.0005$ for each of the cysteamine-pretreated groups compared with the appropriate saline controls.

Cysteamine pretreatment produced a substantial (65.2%) depletion of SRIF in both saline and amphetamine conditioned groups, $F(1,16)=34.4$, $p<0.0005$ (Table 2). SRIF levels in saline-pretreated 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 SRIF 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 suggest that striatal SRIF depletions produced with cysteamine attenuate the motor effects of DA agonists. Vécsei *et al.* [30] have previously reported that intraventricular injections of cysteamine 4 hr before behavioral testing reduces spontaneous locomotor activity. In

agreement with this, we have found that cysteamine given subcutaneously 3 hr before testing reduced locomotor activity during habituation. Amphetamine induced motor stimulation was also attenuated by this treatment 4 hr after cysteamine. That this effect is a result of a modulation of the actions of central DA activity, and not merely a reflection of lower basal activity levels, is supported by the observation that amphetamine stimulated motor activity is still reduced 12 hr after cysteamine injections, a time when basal locomotor activity is not altered.

Vécsei *et al.* [30] reported that activity in an open field was normal 24 hr following intracerebroventricular cysteamine injections. However, SRIF appears to remain depleted up to 72 hr after intrastriatal cysteamine injections [3]. The present results indicate that SRIF is depleted for at least 15 hr following a single subcutaneous cysteamine injection, for 48 hr after a single intra-accumbens cysteamine injection, and for 36 hr after repeated cysteamine treatments. These data suggest that some process such as increased SRIF release or the up-regulation of SRIF receptors [27] may compensate for some of the motor effects seen initially after SRIF depletion.

On the basis of the results of the parenteral injections of cysteamine alone, it is not possible to exclude the possibility that peripheral effects of cysteamine are responsible for the reduction in amphetamine stimulated locomotor activity. The dopaminergic innervation of the NAS is known to mediate amphetamine-induced locomotor behavior [15], and intra-accumbens injections of cysteamine produced a reduction in amphetamine-induced motor activity, indicating a central locus of action. That NAS, but not striatal or cortical SRIF levels were reduced by intra-accumbens injections further specifies the NAS as the site of these locomotor effects of cysteamine.

Peripheral administration of cysteamine, which reduces striatal SRIF, also attenuates the stereotypy produced with apomorphine (Fig. 1). Apomorphine is a direct DA receptor agonist that appears to produce stereotypy via an action in the striatum [15]. This suggests that SRIF may modulate the effects of DA activity post-synaptic to DA-releasing terminals in the striatum. The finding that neither peripheral nor central administration of cysteamine altered DA or DA metabolite levels [2,3] is consistent with this suggestion.

While cysteamine treatment attenuated the motor effects of DA 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 DA agonists are independent of each other [12,17].

It is interesting to note that SRIF was never depleted more than 65% in the present study even with repeated cysteamine treatments. Previous reports have also noted that depletions of central SRIF produced by single injections of cysteamine are not complete [3, 5, 19, 24, 27]. This suggests that SRIF may be differentially compartmentalized; the SRIF in only certain compartments being susceptible to the actions of cysteamine. This hypothesis is supported by our immunohistochemical results in which cysteamine depleted SRIF immunoreactivity from NAS terminal fields, but not cell bodies.

Cysteamine appears to be a useful pharmacological agent with which to study the central actions of SRIF. It is apparent from the present results that SRIF may play a role in modulating the motor effects, but not the reinforcing actions, of DA agonists. Furthermore, it is likely that this modulation occurs postsynaptic to the DA-releasing terminals in the striatum, since SRIF depletions reduce the motor effects of both indirect and direct acting DA receptor agonists, while not appearing to alter DA metabolism.

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