Pharmacology Biochemistry and Behavior, Vol. 2, pp. 79–86. ANKHO International Inc., 1974. Printed in the U.S.A.

Ventricular Infusion of Norepinephrine and Amphetamine: Direct Versus Indirect Action[1]

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(Received 24 October 1973)

SEGAL, D. S., C. MCALLISTER AND M. A. GEYER. *Ventricular infusion of norepinephrine and amphetamine: direct versus indirect action.* PHARMAC. BIOCHEM. BEHAV. 2(1) 79–86, 1974. – The behavioral hyperactivity produced by intraventricular infusions of norepinephrine in rats is potentiated by pretreatment with 6-hydroxydopamine. Pretreatment with the catecholamine uptake blocker desmethylimipramine does not alter the effect of norepinephrine, which indicates that the potentiation observed after 6-hydroxdopamine is due to an enhancement of receptor sensitivity rather than to a loss of an uptake inactivation mechanism. Intraventricular infusions of d-amphetamine result in behavioral effects which are comparable to those observed following systemically administered amphetamine: (1) increasing doses produce a graded increase in locomotor activity; (2) high doses elicit a pattern of stereotypy; and (3) d-amphetamine is more potent than l-amphetamine in producing these behavioral effects. The behavioral effects of amphetamine, in contrast to those induced by norepinephrine, appear to be dependent upon the functional levels of brain catecholamines as indicated by the suppression of amphetamine effects following pretreatment with alpha-methyl-p-tyrosine or reserpine. These results provide evidence for a direct mechanism of action for infused norepinephrine and indicate the potential usefulness of norepinephrine-induced activity as an index of alterations in receptor sensitivity.

Intraventricular infusion Amphetamine Norepinephrine Receptor sensitivity Behavioral activity Stereotypy 6-Hydroxydopamine

INTRAVENTRICULAR infusion of norepinephrine (NE) produces a dose-dependent increase in behavioral arousal [4, 9, 10]. This effect appears to reflect the specific facilitation of central noradrenergic transmission and may be due to the direct action of NE at postsynaptic receptors and/or its indirect effect on noradrenergic terminals (e.g. release of stored NE). That the observed behavioral effect is primarily direct in nature is indicated by previous studies in which pretreatment with alpha-methyl-p-tyrosine (αMT) or reserpine were found to enhance rather than to diminish behavioral activation induced by NE infusion [4]. Since both αMT, which inhibits NE synthesis, and reserpine, which disrupts NE storage, deplete intraneuronal catecholamines (CA), it is unlikely that the infused NE has its effect by the release or displacement of endogenous amines.

In the present series of studies an attempt has been made to further examine the mode of action of infused NE following various pharmacological pretreatments. The results indicate the potential usefulness of this technique as an index of receptor sensitivity.

GENERAL METHOD

Animals

Male Sprague-Dawley rats were obtained from Carworth Farms and weighed approximately 300–350 g.

Surgery

Each animal was implanted with a cannula in the right lateral ventricle (De Groot: A 5.4, ML 2.0, H + 4.0) and housed individually with ad lib access to food and water for at least one week prior to testing. A 12-hr light/dark cycle was maintained.

Procedure

During test sessions the animals were placed in activity chambers and connected to the infusion apparatus (described in detail previously [5,9]) 45 min prior to the activation of the infusion pump. Rats were then infused for 2 hrs with either 0.9% saline, dl-norepinephrine (dl arterenol HCl,

[1] Supported by NIMH Grant MH23209-01.

Calbiochem), or l- or d-amphetamine sulfate (Smith, Kline and French). Pretreatment procedures are described for each experiment where relevant. Activity was measured as cross-overs in chambers whose floors were electronically divided into quadrants. Each animal was infused only once at a constant rate of 20 μl/hr. Following testing animals were infused with Methylene Blue dye and the cannula placements were verified by gross dissection of the brains. The Mann-Whitney U test [11] was used to determine statistical significance unless otherwise indicated.

EXPERIMENT 1

Pretreatments with αMT or reserpine were used in previous studies [4] to examine the role of endogenous CA levels in the behavioral effect of infused NE. Since both pharmacological agents enhanced the NE-induced hyperactivity, it was suggested that infused NE has a direct effect on postsynaptic receptors independent of the level of endogenous NE pools. The increased response to NE was interpreted as possibly reflecting the development of receptor supersensitivity. However, since reserpine and αMT administration decrease endogenous amines without altering the structural integrity of the nerve terminal, it could still be suggested that a small, functional pool of CAs mediates the effects of the infused NE. In order to test this possibility, CA terminals were destroyed by intraventricular administration of 6-hydroxydopamine (6-HD) three weeks prior to infusion with NE. 6-Hydroxydopamine pretreatment would be expected to diminish the behavioral effects of infused NE if those effects were mediated by a small endogenous pool of NE. Since a significant increase in NE-induced hyperactivity was observed, the possible involvement of receptor sensitivity was subsequently examined. Trendelenberg [15] has demonstrated that increases in sensitivity to administered CAs in the peripheral nervous system (e.g. nictitating membrane) may be mediated by decreases in the capacity of the uptake inactivation system as well as by increases in receptor sensitivity. In order to distinguish between these two possible mechanisms, the effects of NE infusions following uptake inactivation by desmethylimipramine (DMI) were examined. It was found that pretreatment with DMI did not alter the behavioral effects of NE infusion.

Method

The procedures were as described above (see General Method) except that one week following cannulation animals were injected with either vehicle (25 μl) or 250 μg 6-HD (HBr, Calbiochem) (dissolved in 25 μl of 0.1% ascorbic acid in 0.9% saline) three weeks prior to infusion with either 0.9% saline or 1.0 μg/μl or 2.0 μg/μl NE. This dose of 6-HD has been shown to produce a rapid and sustained decrease in CA levels [1,16] and in tyrosine hydroxylase activity [16, Segal and Kuczenski, unpublished] in the rat brain, reflecting the destruction of CA neurons.

Additional groups of rats were injected IP with either 25mg/kg DMI (Norpramin, Lakeside) or saline 1 hr prior to a 2 hr infusion with either saline, 0.5 μg/μl NE, or 2.0 μg/μl NE. This dose of DMI has been shown to produce a marked inhibition of NE uptake, both *in vivo* [3] and *in vitro* [6].

Results

Figure 1a shows the effects of saline and NE infusions in 6-HD pretreated rats as the mean percent of appropriate saline pretreated controls. The locomotor activity of animals infused with saline three weeks following 6-HD treatment was not significantly different from control levels. The infusion of either 1.0 μg/μl or 2.0 μg/μl NE resulted in significant increases in the behavioral activity of 6-HD pretreated animals when compared to the levels of activity produced by the same doses of NE infused in saline pretreated rats (increased in the mean percent $\pm$ S.E.M. to 190 $\pm$ 20 and 169 $\pm$ 22, respectively; $p<0.002$ for each). Similar potentiation of NE-induced increases in Sidman avoidance responding was also observed two weeks after 6-HD treatment [Browne, Segal and Geyer, unpublished]. Desmethylimipramine pretreatment was not effective in altering the activity produced by either 0.5 μg/μl or 2.0 μg/μl NE (Fig. 1b).

Discussion

Pretreatment with 6-HD produces a marked potentiation of the NE-induced hyperactivity. These results are consistent with previous studies in which reserpine or αMT was used to alter endogenous NE levels and supports the hypothesis of a direct receptor effect of infused NE. The destruction of NE neurons with 6-HD appears to preclude the possibility of an indirect effect mediated by NE terminals.

These results are especially interesting in view of the frequently reported recovery of behavioral activity following the initial reduction produced by 6-HD treatment [7,14]. In view of such recovery at a time when NE levels and uptake as well as tyrosine hydroxylase activity remain substantially reduced, some investigators have suggested that the behavioral changes produced by 6-HD are not subserved by brain NE pathways. The present results suggest that, at least in part, recovery of function following 6-HD treatment may be mediated by receptor supersensitivity. This model would predict that recovery of function would be limited by the availability of endogenous NE in neurons which survive the 6-HD treatment. Thus, depending upon the dose of 6-HD administered and upon the requirements of NE dictated by the conditions in which the specific behavior is measured, recovery may appear to be complete or partial. Experiments are currently in progress in our laboratory to more completely characterize the behavioral recovery following 6-HD treatment.

The enhanced effect of infused NE and the recovery of function following 6-HD administration may be explained by a mechanism other than receptor supersensitivity. The classic experiments of Trendelenberg and his coworkers [15] indicate that denervation of sympathetic tissue leads to a short latency increase in sensitivity of the denervated tissue to NE and other sympathomimetic agents. This effect appears to be due to the loss of uptake inactivation and to the consequent increased NE in the regions adjacent to the receptor sites (biophase). This increased sensitivity is accompanied by a more gradual enhancement of responsiveness (with a peak at two–three weeks) to applied NE resulting from receptor supersensitivity.

The absence of any effect on NE-induced hyperactivity following DMI pretreatment indicates a relative ineffectiveness of uptake inactivation on the infused NE. The uptake apparatus may be saturated by the continuous infusion of relatively low concentrations of NE. The additional NE made available in the absence of uptake inactivation might

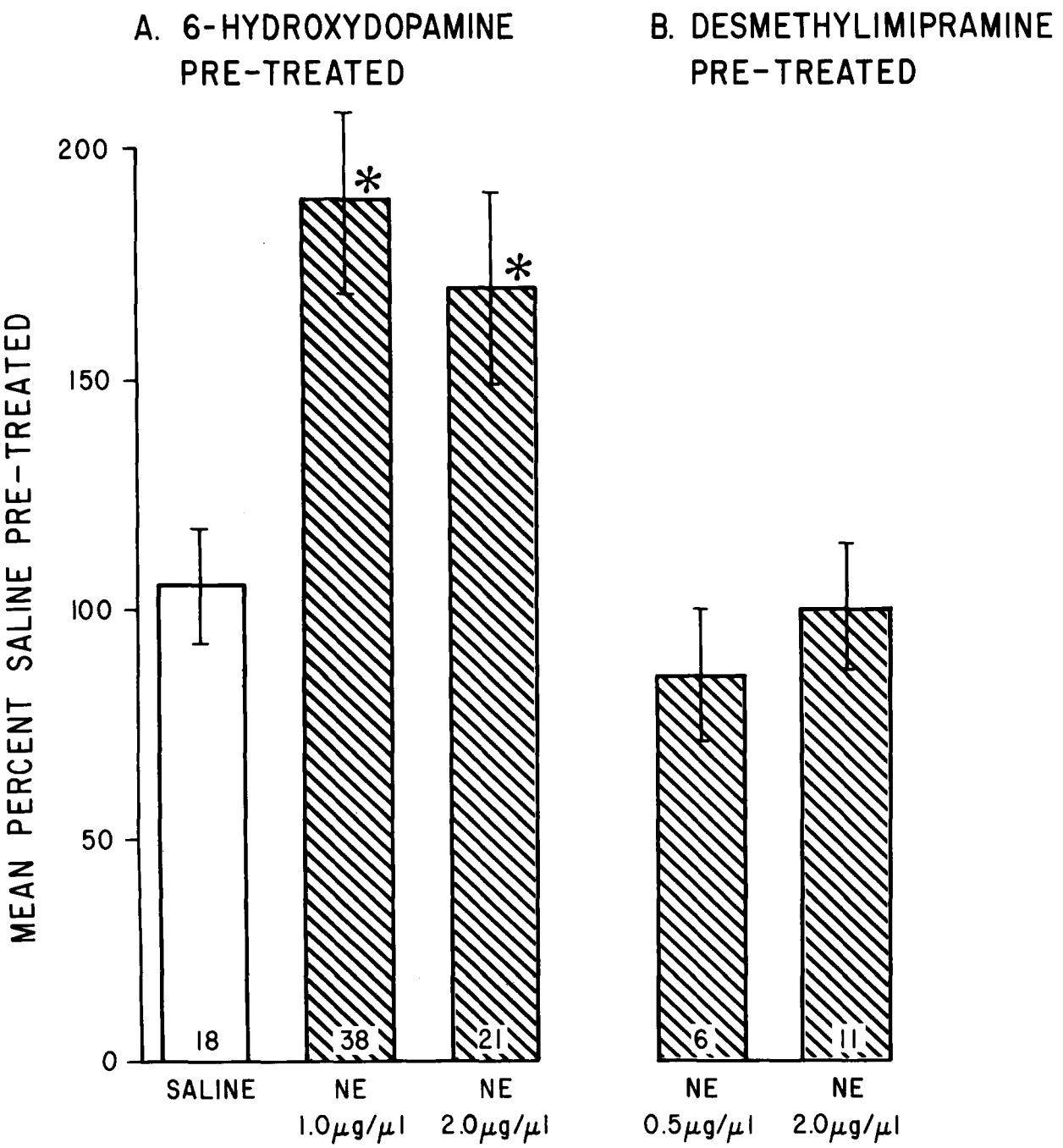

FIG. 1. Cross-overs (± S.E.M.), mean percent of corresponding saline pretreated controls, during 2 hrs of infusion with either isotonic saline or with various doses of NE. A. Pretreatment with 6-HD (250 μg in 25 μl) 3 weeks prior to infusion. 6-HD potentiated the response to infused NE. B. Pretreatment with DMI (25 mg/kg, IP) 1 hr prior to infusion. * Significantly greater than saline pretreated controls, $p<0.002$.

be expected to be small relative to the amounts being infused. Thus, it appears that the augmentation in motor activity produced by infused NE in animals pretreated with 6-HD is due to receptor supersensitivity.

In the following studies the mode of action of infused NE was further examined by comparing it with intraventricularly administered amphetamine following various pharmacological treatments. Amphetamine was chosen because its behavioral effects following systemic administration are similar to those observed during NE infusion [20] and because evidence indicates that the amphetamine effects are mediated by an indirect action, either through release of NE or blockage of its uptake inactivation.

EXPERIMENT 2

Comparison of the effects of amphetamine and NE would be most accurate if both agents were administered by the same route, thus allowing for more similar patterns of drug distribution and rates of accumulation as well as comparison of equi-molar dose ranges. Since NE does not readily pass the blood brain barrier, we first examined the behavioral effects of intraventricularly infused amphetamine preliminary to the comparison of amphetamine with NE. Special attention was directed at amphetamine-induced dose-dependent increases in locomotor activity, the relative potency of d- and l-amphetamine, and the elicitation of stereotypy (e.g. licking, biting, sniffing and gnawing) at high doses of amphetamine. The results indicate that intraventricular and systemic administration of amphetamine produce comparable effects presumably by a similar mechanism of action.

Method

According to the procedures described above, rats were infused with either saline, or 1.0 μg/μl, 2.0 μg/μl, or 3.0 μg/μl d-amphetamine, or 3.0 μg/μl l-amphetamine. In addition to the automatic recording of cross-overs, periodic observations through a viewing lens were made in order to detect the occurrence of amphetamine-induced stereotypies.

Results

Intraventricular infusion of d-amphetamine produced a graded increase in gross locomotor activity over the dose range tested (Kruskal-Wallis test: $H = 24.2$; $df = 3$; $p<0.001$) (Fig. 2). Stereotypy, including sniffing, chewing and biting, was observed during the last half-hour of infusion in several of the animals infused with 2.0 μg/μl d-amphetamine and in most of the animals infused with the highest dose (3.0 μg/μl d-amphetamine). The number of cross-overs produced by 3.0 μg/μl l-amphetamine, although significantly greater than saline controls over the 2 hr session (89 ± 20 and 17 ± 5, respectively; $p<0.001$), was substantially below that produced by 3.0 μg/μl d-amphetamine (274 ± 72; $p<0.01$). No stereotypy was observed with l-amphetamine during the 2 hr test session.

Discussion

The results obtained with intraventricular administration of d-amphetamine indicate that as with systemic administration: (1) increasing doses produce a graded increase in locomotor activity; (2) high doses elicit a pattern of stereotypy, including licking, biting, gnawing and chewing; and (3) d-amphetamine is more potent than l-amphetamine in producing these behavioral effects [8]. It is unlikely that the effects of centrally administered amphetamine are mediated through a peripheral action since the doses used are far below those required to produce comparable behavioral effects with systemic administration. Therefore, it may be assumed that amphetamine or one of its metabolites formed in the brain is responsible for the hyperactivity and stereotypy and that this effect is, at least in part, mediated by an indirect action on CA neurons consonant with the proposed mechanism of action of systemically administered amphetamine (see Sulser and Sanders-Bush [13]).

In the next series of studies an attempt was made to further substantiate the differential effects of NE (direct receptor activation) and amphetamine (indirect action) at CA synapses.

EXPERIMENT 3

If the behavioral effects produced by centrally administered amphetamine are mediated by an indirect action of amphetamine on endogenous pools of NE, then decreases in those pools ought to correspond to a diminished amphetamine effect. In order to test this hypothesis, animals were pretreated with either reserpine, which depletes NE by impairing the vesicular storage mechanism, or by αMT, which inhibits tyrosine hydroxylase, the rate-limiting enzyme in CA biosynthesis [17].

However, whereas reserpine and αMT would be expected to diminish the amphetamine-induced activity, the effects of NE infusion should not be decreased if its action is directly on postsynaptic receptors. In fact, previous results in our laboratory indicate that NE-induced hyperactivity is enhanced following pretreatment with reserpine or αMT [4]. The present results confirm these previous findings and suggest that, as with systemic administration, some of the effects of centrally infused amphetamine are mediated by a presynaptic action of this drug at CA synapses.

Method

Rats were injected with saline or αMT (50 mg/kg. IP) once a day for 8 days or with saline or reserpine (0.5 mg/kg, IP) once a day for 5 days and placed in the activity chambers 2 or 24 hr respectively, following the last injection. Either 1.0 μg/μl NE or 2.0 μg/μl d-amphetamine was infused according to the procedures described above. The doses of NE and amphetamine were chosen for their comparable effects on behavioral activity during the first hour of infusion.

Results

Since the results for the saline control groups were not significantly different, these data were pooled for comparison with the experimental groups. Pretreatment with αMT did not significantly alter behavior either during the preinfusion interval or during the infusion of 1.0 μg/μl NE when compared to saline pretreated controls (Fig. 3). In contrast, the amphetamine-induced hyperactivity was substantially reduced by αMT pretreatment during both the first and second hrs of infusion in comparison to control values (21 ± 5 and 25 ± 8, respectively; 63 ± 13 and 94 ± respectively; $p<0.05$ for hr one and $p<0.02$ for hr two).

Since amphetamine-induced activity during the second hr of infusion in control animals is considerably greater than that produced by NE during a comparable time interval, it might be suggested that a limited range of response reduction (i.e. floor effect) results in an apparent differential effect of αMT on amphetamine and NE. However, the behavioral activity (mean cross-overs ± S.E.M.) in the αMT + NE group was significantly higher than in the αMT + amphetamine group during the first hr (42 ± 6 and 21 ± 5, respectively; $p<0.02$) during which time the level of activity in corresponding controls is similar. Therefore it appears that a floor effect is not present and that the interpretation of qualitatively different effects of αMT on NE- and amphetamine-induced activity is valid.

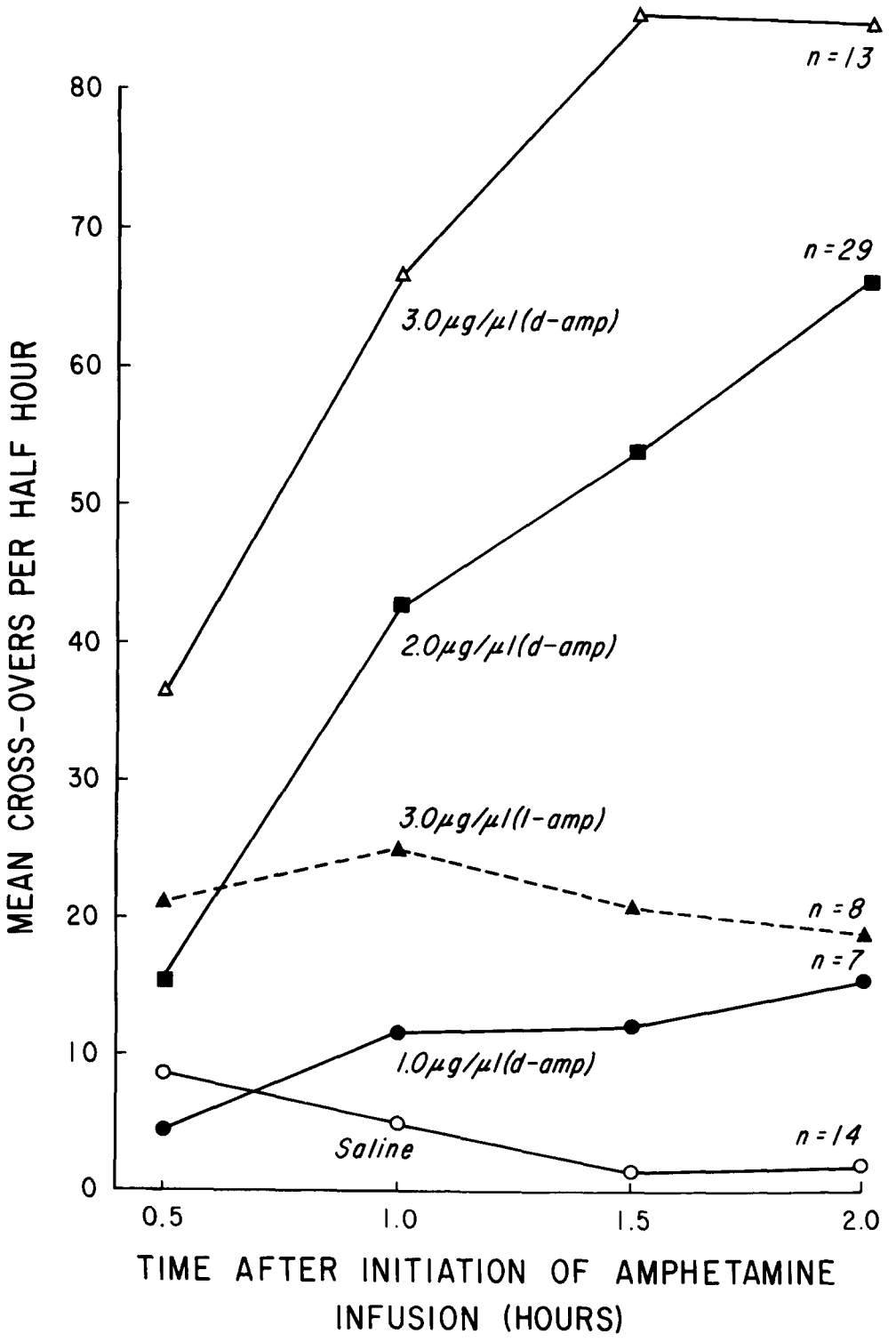

FIG. 2. Mean number of cross-overs during successive 30-min intervals of infusion with isotonic saline, 3.0 μg/μl l-amphetamine, or 1.0 μg/μl, 2.0 μg/μl or 3.0 μg/μl d-amphetamine. D-amphetamine produced a dose-dependent increase in cross-overs. The enhanced activity during infusion of l-amphetamine was substantially below that produced by equimolar doses of d-amphetamine. Stereotypy elicited by the high doses of d-amphetamine is discussed in the text.

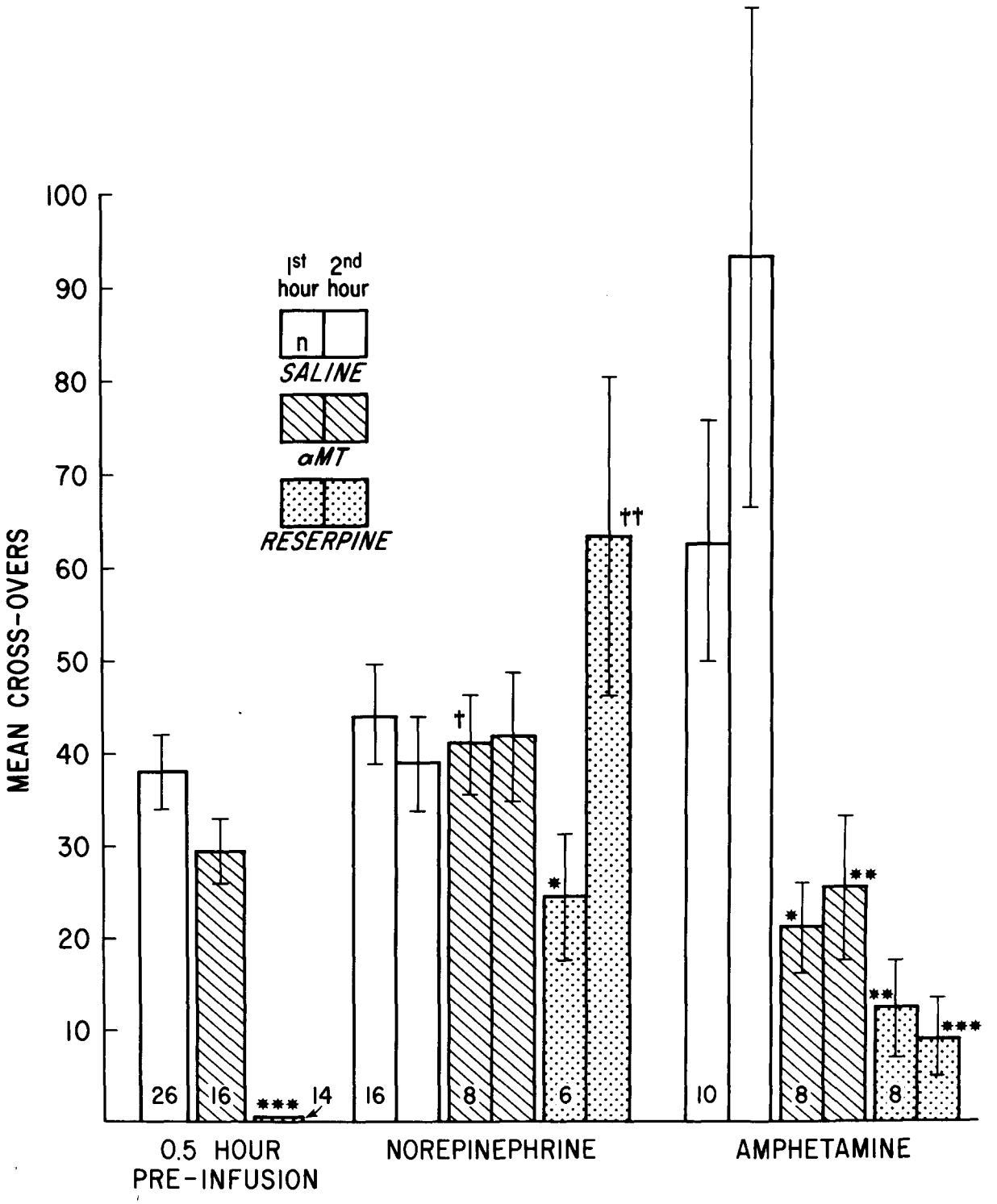

FIG. 3. Mean number of cross-overs (± S.E.M.) during successive hr periods of infusion with 1.0 μg/μl NE or 2.0 μg/μl d-amphetamine after IP injection of saline or αMT (50 mg/kg for 8 days) or reserpine (0.5 mg/kg for 5 days). Behavioral activity was measured 2 or 24 hrs, respectively, following the last injection. Both reserpine and αMT diminished the amphetamine-induced increase in cross-overs.

†Significantly greater than αMT + amphetamine group, $p<0.02$.
††Significantly greater than reserpine + amphetamine group, $p<0.001$.

Unlike αMT, the reserpine pretreatment produced a significant mean reduction in activity (0.4 ± 0.2) as compared to saline controls (38 ± 4; $p<0.002$). The reserpine-induced decrease was partially reversed by NE infusion during the first hr (25 ± 7), although still significantly below corresponding saline pretreated controls (44 ± 5; $p<0.05$). During the second hr of NE infusion activity in the reserpine pretreated animals was comparable to control levels showing, in fact, a mean increase. In contrast, the amphetamine effect was substantially blocked by reserpine treatment (63 ± 14 ± 94 ± 27, as compared to 12 ± 5 ± 9 ± 4; $p<0.02$ and $p<0.002$, respectively). The reserpine and NE group was significantly more active in the second hr than was the reserpine + amphetamine groups ($p<0.01$).

The decrease in motor activity could not be explained as a potentiation of the amphetamine effect which would be expected to enhance stereotypy and reduce locomotion, since no stereotypy was observed during the infusion of amphetamine. Rather, the animals exhibited the typical appearance of reserpine injected rats and were flaccid when removed from the experimental chambers.

Discussion

Both reserpine and αMT pretreatments were found to differentially affect the behavioral activity induced by infusions with amphetamine and NE. The effect of NE infusion was unaltered by αMT and reversed the reserpine-induced depression. These findings substantiate results obtained previously [4] and are consonant with a direct receptor activation by the infused NE. In contrast, the behavioral effects of infused amphetamine appear to be indirect since reduction of endogenous NE levels, either by synthesis inhibition with αMT or impairment of vesicular storage with reserpine, produced a marked reduction in the amphetamine response. These drug interactions are similar to those observed with systemically administered amphetamine. Alpha-methyl-p-tyrosine has been reported by many investigators to abolish the effects of amphetamine [18], and recently, Fibiger, Trimbach and Campbell [2] have shown that the previously reported potentiation of amphetamine effects by reserpine pretreatment [12] is due to the weight loss induced by reserpine. With correction for the effects of weight loss, reserpine was found to decrease the amphetamine augmentation of locomotor activity. That no synergism with respect to hyperactivity was observed between reserpine and intraventricularly administered amphetamine indicates that the reserpine-induced weight loss may: (1) alter the effect of amphetamine on peripheral structures (subsequently being translated into enhanced motor activity); or (2) alter the peripheral metabolism of amphetamine and/or its access to the central nervous system. These effects of weight loss would be circumvented by the central administration of amphetamine. Therefore, on the basis of the results obtained with both αMT and reserpine pretreatments, it appears that at least with respect to some behavioral effects, the actions of amphetamine administered centrally or systemically are comparable. These results are consonant with an indirect mode of action of amphetamine on CA synapses.

GENERAL DISCUSSION

The results obtained indicate that infused NE produces a direct receptor activation: (1) the destruction of NE neurons with 6-HD resulted in a marked potentiation of NE-induced hyperactivity. Similar potentiation could not be obtained with DMI pretreatment, indicating that these effects are due to receptor supersensitivity rather than to the loss of an uptake inactivation mechanism [15]; (2) the behavioral effects of amphetamine administered either systemically or centrally are comparable and appear to be dependent upon the functional levels of brain CAs as indicated by suppression of activity following αMT or reserpine pretreatment. These findings are similar to the results obtained with the infusion of dopamine (DA) which also appears to produce behavioral activity indirectly through its effect on NE neurons, possibly by conversion to NE. In previous studies we have found that DA-induced hyperactivity could be abolished by pretreatment with imipramine [5], which blocks the uptake of DA into NE terminals or reserpine which blocks conversion of DA to NE [4].

In contrast to the amphetamine and DA effects, the behavioral activity induced by NE is not diminished by reductions in endogenous CA levels, which is consistent with a primary receptor action for infused NE. Additional studies are currently in progress to further substantiate the possible direct mechanism of NE action and to explore the use of NE-induced activity as an index of alterations in receptor sensitivity following long-term drug administration. These investigations may provide some insight into the role of receptor sensitivity in the development of drug tolerance.

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