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Chronic Amphetamine: Is Dopamine a Link In or a Mediator of the Development of Tolerance and Reverse Tolerance?

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KUCZENSKI, R. AND N. J. LEITH. *Chronic amphetamine: Is dopamine a link in or a mediator of the development of tolerance and reverse tolerance?* PHARMAC. BIOCHEM. BEHAV. 15(3) 405–413, 1981.—Rats were administered chronic multiple injections of amphetamine (AMPH) using dosage regimens which produce tolerance to the AMPH facilitation of self-stimulation responding, or reverse tolerance (sensitization) to the locomotor stimulant and stereotypy-producing effects of the drug. Subsequently rats were challenged with AMPH at behaviorally relevant doses and times and striatal and mesolimbic dopamine (DA) dynamics were assessed using the conversion of ^{3}H-tyrosine to ^{3}H-DA, and endogenous levels of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) as indices of dopaminergic function. Acute administration of AMPH produced dose and time related changes in all indices of DA function in both the striatal and mesolimbic brain regions. Co-administration of haloperidol during chronic AMPH pretreatment prevented the appearance of most of the behavioral changes induced by chronic AMPH, suggesting an important role for DA systems. However, following chronic AMPH treatment, no additional biochemical changes in striatal or mesolimbic DA metabolism could be detected which would parallel the development of tolerance to AMPH facilitation of self-stimulation behavior or reverse tolerance to AMPH as reflected in enhanced post-stereotypy locomotor activity or a suggested increased intensity of stereotypy. Challenge with AMPH after chronic AMPH pretreatment did accelerate the changes in striatal but not mesolimbic DA metabolism, correlating with the more rapid onset of stereotypy induced by chronic AMPH. Thus, while DA systems appear to be a critical link, not only in the acute effects of AMPH, but also in the development of tolerance and reverse tolerance, most of the behavioral differences between acutely and chronically treated animals are not reflected by comparable differences in DA synthesis and metabolism.

Amphetamine Tolerance and reverse tolerance Chronic administration Dopamine

CHRONIC administration of amphetamine (AMPH) to rats produces qualitatively distinct behavioral changes depending on the particular behavior which is monitored. The locomotor stimulation and stereotypy produced by the drug exhibit reverse tolerance, characterized by increased locomotor activity, more rapid onset of stereotypy, a suggested heightened intensity of stereotypy, and a pronounced post-stereotypy hyperactivity phase [21,31]. In contrast, the drug's effects in a self-stimulation, discriminative stimulus or self-administration paradigm exhibit tolerance after chronic administration [1, 20, 21, 23]. Although the behavioral effects that result from chronic AMPH pretreatment have been extensively characterized (see preceding paper in this volume, [21]), little data pertaining to their biochemical bases exist, and the neurochemical substrates for tolerance and reverse tolerance remain unidentified.

There is a substantial body of literature suggesting that dopaminergic mechanisms play a crucial role in the locomotor stimulant and stereotypy-producing effects of AMPH (see [5,14] for reviews). For example, lesions of the substantia nigra [10,28] or the mesolimbic dopaminergic system [8,27] can abolish or modify the locomotor stimulant effects of the drug. Disruption of the nigro-striatal dopaminergic pathway also effectively blocks amphetamine stereotyped behavior [9, 10, 12]. Similarly, dopamine appears to be significantly involved in the rewarding effects of AMPH as reflected in self-stimulation behavior [6], discriminative stimulus studies [37] and self-administration of the drug [30].

The consequences of acute AMPH administration on brain DA have been extensively characterized in terms of drug-induced changes in DA synthesis [4, 16, 26] and DA metabolism [17, 29, 36] as well as in changes in dopaminergic neuronal activity [3,13]. In contrast few studies have examined DA dynamics as a function of multiple AMPH administrations, and most of those studies have utilized much higher dosage regimens and longer exposure periods than are necessary to produce either tolerance or reverse tolerance [11,22]. Thus, although chronic AMPH can clearly produce

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changes in DA dynamics, the relevance of such changes to the behavioral changes designated as tolerance and reverse tolerance is uncertain.

We therefore attempted to assess potential changes in transmitter dynamics in both the striatal and mesolimbic DA pathways as a consequence of behaviorally relevant dosage regimens and times after drug challenge, as detailed in the preceeding communication [21]. Because of the complexity of AMPH's interaction with the DA neuron and the potential for ambiguity in the interpretation of biochemical data after AMPH administration when a single measure of DA dynamics is used, we monitored the conversion of intraventricularly administered ^{3}H-tyrosine to ^{3}H-DA, as well as the levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). As a prelude, we first determined whether the changes in locomotor activity and stereotypy produced by repeated AMPH administration (reverse tolerance) could be prevented by pretreatment with a DA receptor blocker, haloperidol, since Barrett and White [2] have already shown that such pretreatment prevents the development of tolerance.

METHOD

Animals and Drug Regimens

Male Sprague-Dawley rats (250–300 g) were obtained from Harlan Industries, Indianapolis, IN and housed singly under standard laboratory conditions with ad lib access to food and water. Animals were maintained under these conditions for one week prior to initiation of any experimental manipulations. All drugs were administered subcutaneously and doses are expressed as the free base unless otherwise specified.

The 1X daily regimen, which produces reverse tolerance, consisted of daily (8:00 a.m.) injections of 3 mg/kg AMPH for six days. Two days following the last injection, animals were administered the challenge drug and either tested behaviorally or sacrificed for biochemical studies. The 3X daily regimen which produces both tolerance and reverse tolerance consisted of three injections daily (8:00 a.m., 2:00 p.m., 8:00 p.m.) for four days beginning with a dose of 1 mg/kg AMPH (as the salt) and incrementing by 1 mg/kg at each subsequent injection. Approximately 48 hr after the last injection of 12 mg/kg, the challenge drug was administered.

Materials

L-[2,6-^{3}H] Tyrosine (49 Ci/mmole) was obtained from Amersham Searle and was purified by adsorption on and elution from Dowex 50X-4(H+) and by passage over alumina, followed by lyophilization. L-Tyrosine, dopamine hydrochloride, and S(+)-amphetamine sulfate were products of Sigma Chemical Company, St. Louis, MO. Haloperidol was a product of McNeil Labs, Fort Washington, PA.

Intraventricular Cannulation

For purposes of intraventricular [^{3}H]tyrosine administration, animals were anesthetized with 40 mg/kg sodium pentobarbital and implanted according to the atlas of König and Klippel [15] at co-ordinates A, 6.1 mm, L, 1.5 mm, and D.V.+2.0 mm. The stainless steel guide cannulae (Plastics Products Company, Roanoke, VA) consisted of 22 g stainless steel tubing mounted on a threaded plastic top. The injection cannula (28 g stainless steel tubing) was cut 1.0 mm longer than the implanted cannula. All cannulae and styluses were beveled at a 45° angle to incise rather than tear neural tissue. Following surgical procedures, the animals were housed individually for at least one week prior to subsequent experimental manipulations.

Behavioral Procedures

AMPH-induced locomotor stimulation and stereotypy were monitored in fully automated symmetrical Y-mazes as detailed in the accompanying study [21]. Briefly, each arm of the maze had a photocell mounted 14 cm from the entrance to the arm. Entering the arm registered one activity count. Thus, this activity measure reflects locomotion from one arm to another.

Biochemical Procedures

DA turnover in striatum was assessed as previously described [16]. Animals received AMPH or saline and fifteen minutes before sacrifice received an intraventricular injection of 10 μCi L-[2,6-^{3}H]tyrosine in saline in a volume of 2.0 μl. At the time of injection a 38 cm length of polyethylene tubing attached to a micrometer syringe was screwed to the plastic top and the injection volume was delivered over a 10 sec time period. The injection cannula remained in place an additional 30 sec to allow for diffusion to occur. Rats were killed by decapitation, corpora striata were removed, weighed, and homogenized in 7 ml 0.4 N perchloric acid containing 0.5% sodium metabisulfite. The samples were centrifuged and the supernatant was adjusted to pH 8.2 with 2.5 N Tris base and added to 200 mg alumina. The supernatant from the alumina extraction was retained as the tyrosine fraction, and DA was eluted from the alumina with 0.2 N HCl. The tyrosine fraction was adjusted to pH 1.5 with HCl, and further purified by passage over a column (0.5 cm×5.0 cm) of Dowex 50-X4 (Na^+), 200–400 mesh, previously equalibrated with 0.1 M Na phosphate buffer, pH 6.5 containing 0.5% sodium metabisulfite and 0.1% EDTA. Tyrosine was eluted from the column with 0.1 M Na_3PO_4. Tyrosine and DA were assayed fluorometrically according to the procedures of Udenfriend [34] and Laverty and Taylor [19], respectively. Radioactivity was determined in ACS (Amersham-Searle) (enriched with 75 mg/l POPOP) on a Searle Analytic, Inc., Mark III liquid scintillation counter. Recoveries of tyrosine and DA were determined on tissue samples to which had been added known amounts of unlabeled DA and tyrosine. Calculation of the conversion index was performed according to Costa *et al.* [7].

Alternatively, DA dynamics were assessed in striatum and the mesolimbic region (containing the nucleus accumbens and tuberculum olfactorium) by monitoring levels of DA and its metabolites DOPAC and HVA. DOPAC and HVA were isolated by a non-automated modification of the method of Westerink and Korf [35] as previously described [17]. DOPAC was assayed with modifications [17] of the procedures of Spano and Neff [32] and Westerink and Korf [36]. HVA was assayed according to Westerink and Korf [36]. Recoveries for DOPAC and HVA were 88±2% and 84±3%, respectively. For both metabolites, 15 ng yielded fluorescence values twice the blank.

Statistics

Data were analyzed using t-tests except in cases involving multiple groups within a single experiment when analysis of variance was used.

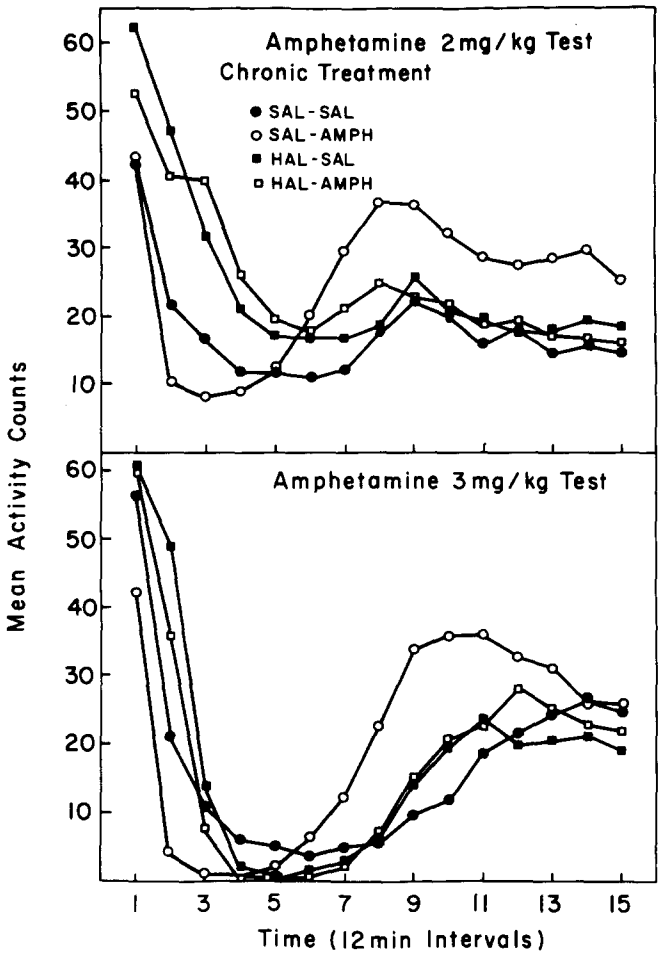

FIG. 1. The effects of chronic pretreatment with AMPH and/or HAL on locomotor activity of rats in response to challenge with AMPH. Rats were pretreated with saline or 0.2 mg/kg HAL followed 15 min later with saline or 3 mg/kg AMPH for six days. Two days following the last pretreatment injection, rats were administered 2 mg/kg AMPH (upper panel) or 3 mg/kg AMPH (lower panel) and their activity was recorded for three hr.

RESULTS

Effect of Haloperidol Pretreatment on Chronic AMPH-Induced Changes in Locomotor Activity and Stereotypy

To obtain some evidence that the changes in AMPH induced locomotor activity and stereotypy following chronic pretreatment with the drug are dependent on dopamine sensitive mechanisms, animals were pretreated with the DA receptor blocker haloperidol (HAL) prior to each daily injection with AMPH. Preliminary experiments indicated that 0.2 mg/kg HAL provided a functional blockade of striatal and mesolimbic DA receptors [17] and fully prevented the behavioral response to an acute administration of 3 mg/kg AMPH (data not shown). Therefore, animals were administered 0.2 mg/kg HAL or vehicle and fifteen minutes later received 3 mg/kg AMPH or vehicle daily for six days. Forty-eight hr after the last injection, rats were challenged with 2 or 3 mg/kg AMPH and their activity was monitored for 3 hr. Results are presented in Fig. 1. Analysis of variance revealed a significant interaction of HAL pretreatment with the chronic AMPH treatment and time, $F(14,728)=2.7, p<0.001$. As can be seen from the data, this interaction occurs because chronic administration of AMPH resulted in a more rapid onset to stereotypy and enhanced post-stereotypy locomotor activity compared to saline pretreated animals when challenged with 2 or 3 mg/kg AMPH. In contrast, those animals pretreated with HAL alone or HAL plus AMPH presented a delayed onset to stereotypy and exhibited no enhancement of locomotion during the post-stereotypy activity phase. Thus, HAL pretreatment during the period of chronic AMPH administration prevented the development of reverse tolerance to AMPH.

Effects of Two Chronic AMPH Regimens on Striatal and Mesolimbic Baseline DA Dynamics

The 3X daily chronic AMPH regimen decreases the baseline response of animals to self-stimulation [20,21]. In contrast, neither regimen alters the locomotor activity of animals in response to saline challenge ([31] and unpublished data from these labs). Therefore, we first determined whether there were changes in baseline DA function following chronic AMPH that might be reflected in striatal and mesolimbic DOPAC and HVA levels and striatal DA levels and conversion index after each of the chronic AMPH regimens. Forty-eight hr after the last of the chronic drug injections, animals were administered saline (1 ml/kg) and were sacrificed 15 min later. The data from several experiments are summarized in Table 1. Neither regimen altered baseline levels of DOPAC or HVA in striatal or mesolimbic tissue, nor were striatal DA levels altered. Both regimens caused a small (11–18%) but statistically significant increase in striatal conversion of ^{3}H-tyrosine to ^{3}H-DA. A statistically significant increase is only observed when the number of animals is large, although a trend toward higher values after the chronic regimen is consistently obtained (see, for example, Table 2).

Effect of 3X Daily AMPH Regimen on Striatal Conversion Index and DA Levels After AMPH Challenge

Both the 3X daily AMPH regimen and the 1X daily AMPH regimen yield identical reverse tolerance to the locomotor activity and stereotypy effects of AMPH challenge, whereas only the former produces significant tolerance to the AMPH facilitation of self-stimulation behavior [21]. To maximize the opportunity of detecting changes in striatal response to AMPH challenge which might parallel the behavioral changes induced by the chronic drug treatment, animals were subjected to the 3X daily regimen and subsequently the striatal conversion index response to AMPH challenge was assessed. Challenge doses and times which parallel distinct behavioral effects of the chronic drug regimen were utilized: 0.5 mg/kg 31 min prior to sacrifice to reflect enhanced locomotor activity and/or tolerance during self-stimulation behavior; 2 mg/kg at 31 min to reflect the more rapid onset of stereotypy; and 5 mg/kg at 90 min to reflect the suggested greater intensity of the stereotypy response. The results are summarized in Table 2. No differences in striatal DA levels or striatal conversion index values between the acute AMPH response or the response after the 3X daily regimen were detected.

TABLE 1

EFFECT OF CHRONIC AMPH REGIMENS ON BASELINE STRIATAL AND MESOLIMBIC DA DYNAMICS

Pretreatment	DOPAC (μg/g)	HVA (μg/g)	DA (μg/g)	CI (nmoles/g/hr)
		Striatum		
Saline	2.21 ± 0.07 (10)	1.24 ± 0.07 (10)	10.40 ± 0.16 (10)	27.6 ± 2.3 (13)
3× daily AMPH	2.08 ± 0.06 (10)	1.17 ± 0.03 (10)	9.83 ± 0.29 (10)	30.7 ± 1.4* (13)
1× daily AMPH	2.21 ± 0.06 (10)	1.26 ± 0.06 (10)	10.57 ± 0.39 (10)	32.7 ± 1.4† (13)
		Mesolimbic		
Saline	1.15 ± 0.03 (10)	0.68 ± 0.04 (10)	—	—
3× daily AMPH	1.11 ± 0.01 (10)	0.67 ± 0.04 (10)	—	—
1× daily AMPH	1.14 ± 0.05 (10)	0.69 ± 0.04 (10)	—	—

Animals received saline or AMPH daily as noted in Method. Forty-eight hrs after the last injection, animals received saline, and fifteen min later were sacrificed. Values are the mean ± S.E.M. Numbers of animals are noted in parentheses.

*$p<0.01$; †$p<0.004$.

Effect of 3X Daily AMPH Regimen on Striatal and Mesolimbic DOPAC and HVA Levels

As an alternative index of CNS DA metabolism, levels of DOPAC and HVA were assessed in striatal and mesolimbic regions in response to challenge with AMPH. Animals were adminstered the 3X daily AMPH pretreatment regimen or saline, and then challenged with 1 mg/kg or 3 mg/kg AMPH thirty min prior to sacrifice. Striatal and mesolimbic DOPAC and HVA levels are presented in Table 3. Analysis of variance revealed a significant effect of pretreatment in striatum only on both DOPAC and HVA levels in response to AMPH challenge, DOPAC: $F(1,20)=8.1$, $p=0.01$; HVA: $F(1,20)=4.5$, $p=0.044$. Thus 3X daily chronic AMPH enhanced the AMPH-induced decline in striatal DOPAC and HVA levels.

Effect of 1X Daily Chronic AMPH on Striatal and Mesolimbic DA Metabolites in Response to Challenge AMPH

Since the enhanced decline in striatal DOPAC and HVA in response to challenge with AMPH after the 3X daily chronic regimen paralleled the more rapid onset of stereotypy, we sought to determine whether a similar biochemical effect would be induced by the 1X daily AMPH regimen since it also produces the behavioral effect. Groups of animals pretreated with 1X daily chronic AMPH or saline were challenged with 0, 1, 2, or 3 mg/kg AMPH 30 min prior to sacrifice and striatal and mesolimbic DA metabolites were assessed. The results are presented in Figs. 2 and 3. Mesolimbic DOPAC and HVA responded identically to AMPH challenge with and without chronic AMPH pretreatment (Fig. 2). In contrast, analysis of variance revealed a significant effect of AMPH pretreatment on the AMPH-induced decline in striatal DOPAC and HVA (Fig. 3), DOPAC: $F(1,30)=6.4$, $p=0.01$; HVA: $F(1,30)=5.2$, $p=0.03$. No change in the response of striatal DA levels to AMPH challenge as a consequence of chronic AMPH pretreatment was detected.

Following chronic AMPH, a challenge dose of 3 mg/kg AMPH produces, as a function of time, the entire constella-

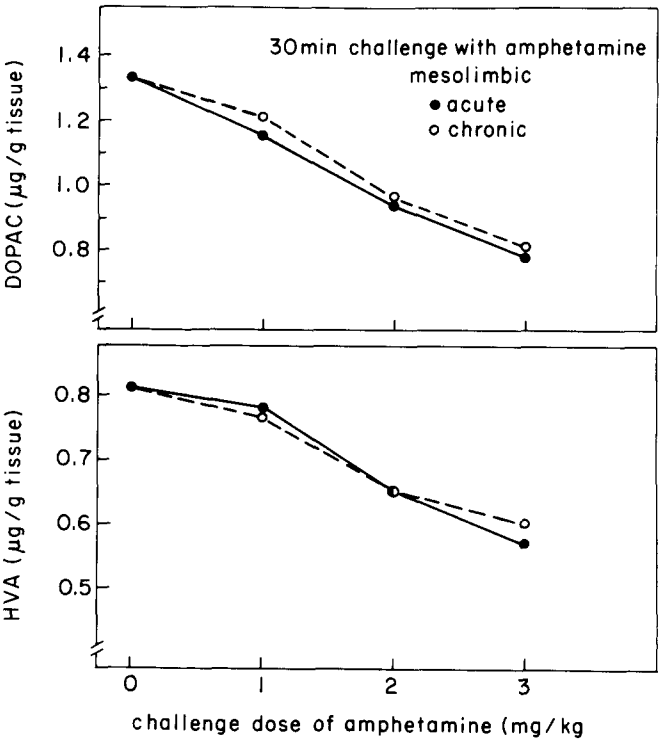

FIG. 2. Effects of challenge doses of AMPH on mesolimbic DOPAC and HVA levels with and without chronic AMPH pretreatment. Rats were administered 3 mg/kg AMPH or saline daily for 6 days. Two days following the last pretreatment injection, rats were administered various doses of AMPH and sacrificed 30 min later. No significant effects of chronic AMPH pretreatment were detected.

TABLE 2

EFFECTS OF 3× DAILY CHRONIC AMPH ON STRIATAL CONVERSION INDEX RESPONSE TO AMPH CHALLENGE

AMPH challenge	Treatment chronic/acute	CI	DA
0.50 mg/kg; 31 min	saline/AMPH	155.8 ± 4.3*	118.6 ± 5.3*
	AMPH/saline	112.6 ± 7.1	100.1 ± 3.8
	AMPH/AMPH	161.5 ± 9.8*	124.9 ± 6.1*
2 mg/kg; 31 min	saline/AMPH	116.2 ± 8.1	127.6 ± 4.1*
	AMPH/saline	111.1 ± 8.2	97.8 ± 3.8
	AMPH/AMPH	119.1 ± 7.7	123.1 ± 3.9*
5 mg/kg; 90 min	saline/AMPH	71.1 ± 10.8*	134.7 ± 4.0*
	AMPH/saline	108.9 ± 6.4	96.3 ± 4.1
	AMPH/AMPH	64.0 ± 9.4*	138.4 ± 4.2*

Animals were administered saline or AMPH utilizing the 3× daily regimen detailed in Method. Forty-eight hrs after the last pretreatment injection animals were administered saline or AMPH as noted in the Table and sacrificed. Values are the mean ± S.E.M. for 6–8 animals, expressed as percent of saline/saline controls.

*Significantly different from saline/saline controls.

tion of behavioral responses that has been designated as reverse tolerance: more rapid onset of stereotypy, an apparent greater intensity of the stereotyped response, and enhanced post-stereotypy locomotor activity. Therefore, in a series of experiments, we examined the striatal and mesolimbic response to challenge with 3 mg/kg as a function of time after challenge with and without 1X daily chronic AMPH pretreatment. The results of these experiments are summarized in Figs. 4 and 5. Mesolimbic DOPAC and HVA responded to challenge AMPH identically at all time points with and without chronic AMPH pretreatment (Fig. 4). In the striatum (Fig. 5), only at 15 min after the challenge dose was a significant effect of chronic AMPH pretreatment detected in DOPAC and HVA levels. At that time point the effect of pretreatment was to enhance the decline in both DOPAC and HVA induced by AMPH challenge, but no change in the maximal decline nor in the recovery from the decline was produced by chronic AMPH pretreatment. Similarly, the maximal decline in striatum induced by 5 mg/kg (from 1.97±0.03 to 1.06±0.05 μg/g in saline pretreated animals) was unchanged by chronic AMPH pretreatment (to 0.99±0.06 μg/g in 1X daily chronic AMPH pretreated animals).

TABLE 3

EFFECT OF 3× DAILY CHRONIC AMPH ON REGIONAL DOPAC AND HVA LEVELS THIRTY MINUTES AFTER CHALLENGE INJECTIONS

Treatment chronic/acute	DOPAC μg/g	HVA μg/g
	Striatum	
saline/saline	1.83 ± 0.07	0.81 ± 0.11
1 mg AMPH	1.33 ± 0.06	0.71 ± 0.07
3 mg AMPH	0.98 ± 0.04	0.53 ± 0.02
AMPH/saline	1.83 ± 0.09	0.79 ± 0.10
1 mg AMPH	1.16 ± 0.05	0.58 ± 0.02
3 mg AMPH	0.88 ± 0.03	0.47 ± 0.02
	Mesolimbic	
saline/saline	0.94 ± 0.03	0.66 ± 0.03
1 mg AMPH	0.71 ± 0.04	0.60 ± 0.04
3 mg AMPH	0.59 ± 0.04	0.51 ± 0.03
AMPH/saline	0.90 ± 0.03	0.68 ± 0.04
1 mg AMPH	0.71 ± 0.05	0.61 ± 0.03
3 mg AMPH	0.59 ± 0.03	0.53 ± 0.04

Animals were pretreated with AMPH 3× daily as described in Method and challenged with AMPH or saline 48 hrs after the last pretreatment injection. Thirty min later animals were sacrificed. Values represent the mean ± S.E.M. for six rats.

Chronic AMPH significantly enhanced the AMPH-induced decrease in striatal DOPAC and HVA by ANOVA (DOPAC: $F(1,20)=8.1$, $p=0.01$; HVA: $F(1,20)=4.5$, $p=0.044$).

DISCUSSION

There is a substantial body of literature, briefly described in the introduction, suggesting a crucial role for DA systems in the locomotor stimulant and stereotypy effects of AMPH, as well as in the rewarding effects of the drug as reflected in self-stimulation, discriminative stimulus, and self-administration studies. Acute AMPH administration at doses which are relevant to these behavioral effects profoundly alters brain DA metabolism and DA transmission. Costa *et al.* [7] first reported the apparent relationship between low dose AMPH stimulation of locomotor activity and changes in DA biochemistry. Subsequently, a variety of

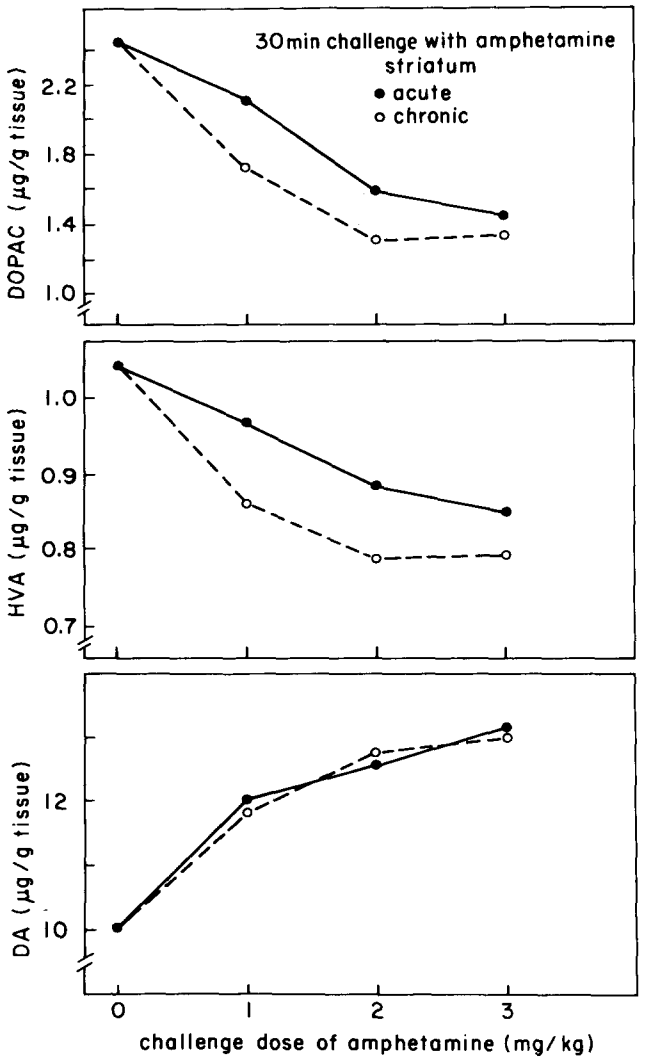

FIG. 3. Effects of challenge doses of AMPH on striatal DOPAC, HVA, and DA levels with and without chronic AMPH pretreatment. See legend to Fig. 2 for further details. Chronic AMPH pretreatment significantly altered the response of striatal DOPAC and HVA, but not DA, to the challenge drug. (Analysis of variance: DOPAC: $F(1,30)=6.4$, $p=0.01$; HVA: $F(1,30)=5.2$, $p=0.03$).

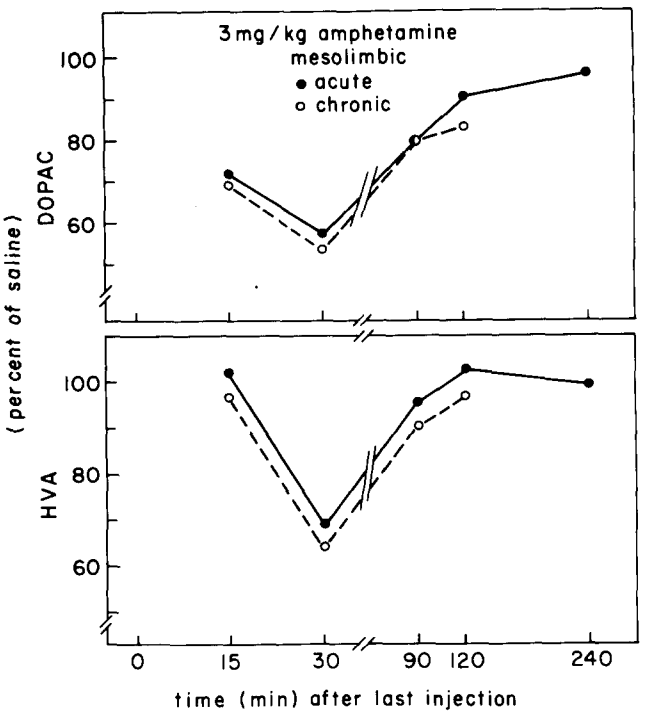

FIG. 4. Effects of 3 mg/kg AMPH at various times on mesolimbic DOPAC and HVA with and without chronic pretreatment with AMPH. Rats were administered 3 mg/kg AMPH or saline daily for six days. Two days following the last pretreatment injection, rats were administered 3 mg/kg AMPH or saline and were sacrificed at various times later. No significant effects of chronic AMPH pretreatment were detected.

investigators have extensively characterized the interaction of AMPH with the dopaminergic neuron. The data are consistent with the suggestion that changes in DA biochemistry may be crucial to the behavioral changes produced by AMPH.

It might therefore be anticipated that pretreatment of animals with chronic AMPH, which markedly alters the subsequent behavioral responses to AMPH, would also alter the biochemistry of DA neurons. Few studies, however, have dealt with alterations in DA dynamics as a function of multiple AMPH administrations. Several investigators have reported decreases in DA levels as a consequence of AMPH pellet implantation [11] or the constant availability of the drug in food or drinking water [22]. That these decreases in DA levels as a consequence of these dosage regimens are not relevant to the behavioral phenomena of tolerance or reverse tolerance is apparent from our failure to observe any decrease in DA levels after chronic AMPH pretreatment (Table 1). The daily administration of 3 mg/kg AMPH for 6 days fully induces the more rapid onset of stereotypy, the apparent increased intensity of the stereotyped response as reflected by the complete absence of locomotor activity, and the enhanced post-stereotypy locomotor activity associated with reverse tolerance [21]. Similarly the 3X daily AMPH regimen produces both reverse tolerance in the locomotor response, and tolerance to the AMPH facilitation of self-stimulation responding. Yet neither regimen alters striatal DA levels. Thus, the biochemical substrates of these behavioral phenomena are unrelated to changes in DA levels.

In fact, the biochemical characterization of striatal and mesolimbic DA neurons presented herein is remarkable in that relatively few changes are effected by either chronic regimen. These data are also consistent with recent electrophysiological data of Staunton *et al.* [33] demonstrating no change following chronic AMPH administration in either the spontaneous firing rate of substantia nigra DA neurons or in the dose of AMPH needed to reduce that firing rate by 50%. AMPH can interact with the dopaminergic neuron at a variety of sites such that it is frequently difficult to specify whether a change in DA biochemistry reflects a direct effect

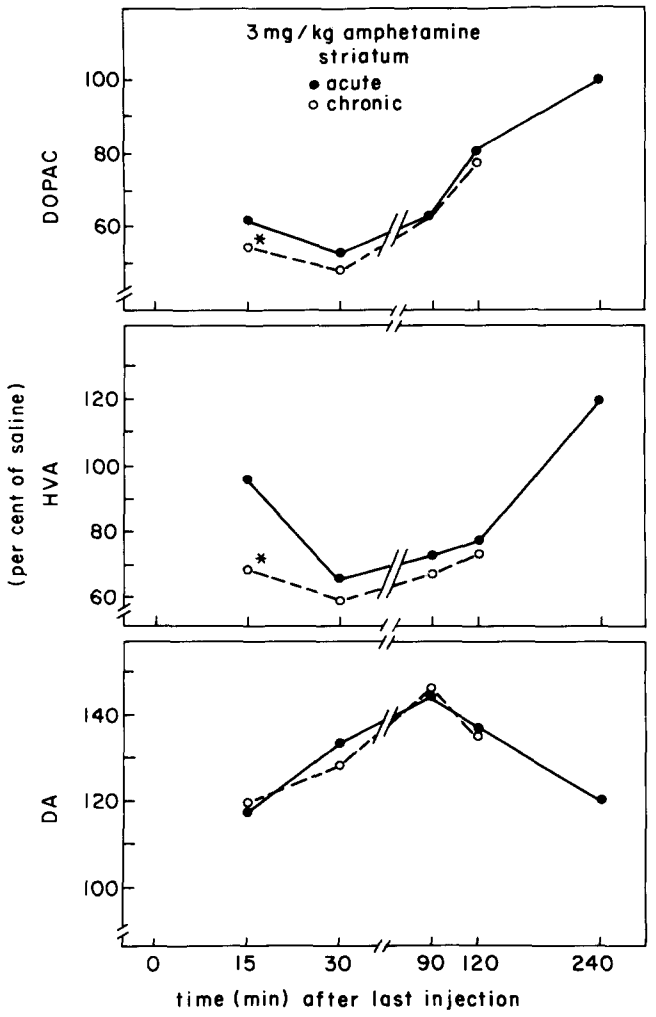

FIG. 5. Effects of 3 mg/kg AMPH at various times on striatal DOPAC, HVA and DA levels with and without chronic AMPH pretreatment. See legend to Fig. 4 for further details. Chronic AMPH significantly increased the decline in DOPAC and HVA only at 15 min after challenge with AMPH (*$p<0.05$ by t-test).

of the drug (e.g. inhibition of monoamine oxidase) or reflects a change in neuronal function. Thus to properly characterize the consequences of chronic AMPH administration on dopaminergic function, a multiple measures approach involving assessment of DA synthesis and metabolism was utilized. Further, these measures were applied as a function of dose of and time after AMPH challenge to correspond with specific behavioral changes induced by the chronic regimens. In particular, we sought to answer three questions: Does chronic AMPH alter baseline DA biochemistry, corresponding to the decrease in baseline self-stimulation responding seen with the 3X daily regimen? Are there changes in DA dynamics at doses and times when the enhanced level of post-stereotypy locomotor activity is evidenced? Do changes in DA dynamics underlie the accelerated onset and apparent increased intensity of the stereotypy response following chronic AMPH?

The data presented in Table 1 indicate that no substantial changes in baseline DA biochemistry occur in striatal or mesolimbic DA neurons as a consequence of chronic AMPH pretreatment. Levels of DA and the metabolites DOPAC and HVA remain identical with and without chronic AMPH pretreatment utilizing either the 1X daily or 3X daily drug regimens. In addition, the small increase in striatal conversion index obtained with both pretreatment regimens does not correlate with the depressed baseline responding of animals in self-stimulation studies after chronic AMPH, since only the 3X daily regimen produces the behavioral effect [21].

Further, no biochemical correlates to the suggested increased intensity of the stereotyped response or the enhanced level of post-stereotypy locomotor activity after chronic AMPH pretreatment were observed in striatal or mesolimbic DA dynamics. Thus, the maximum declines in striatal conversion index at 5 mg/kg AMPH (Table 2) and in striatal or mesolimbic DOPAC and HVA with 3 mg/kg (Figs. 4 and 5) or 5 mg/kg AMPH (see results) were identical with or without chronic AMPH pretreatment. Similarly, the rates of recovery of DOPAC, HVA, and DA levels from their points of maximum change were again identical with or without AMPH pretreatment. Thus, at those points corresponding to times when these behavioral consequences of chronic AMPH are evident—apparent more intense stereotyped response (30–90 min) and enhanced post-stereotypy locomotor activity (90–120 min)—no biochemical consequences of the chronic AMPH pretreatment could be detected, either in striatal or mesolimbic tissue samples.

However, both chronic AMPH pretreatment regimens influenced the initial AMPH-induced decline in DOPAC and HVA in striatum (Table 3, Fig. 3). The effect of chronic AMPH was to enhance the decline in metabolite levels, an effect which might be interpreted as a shift in the dose response curve such that lower doses of challenge AMPH produce a greater effect. Analysis of the response of DA metabolite levels to AMPH as a function of time (Figs. 4 and 5) suggests that the enhanced decline is limited only to very early time points, paralleling the more rapid onset of stereotypy induced by chronic AMPH pretreatment. Further, this effect of chronic AMPH was not obtained in the mesolimbic region, but rather was confined to the striatum.

The decline in striatal DOPAC and HVA levels following acute administration of AMPH achieves a maximum at a dose near 3 mg/kg and at higher doses the maximum decline occurs within 15 min [16], thus paralleling the dose and time response curves for the onset of focused stereotypy, and the dose response curve for AMPH inhibition of nigro-striatal neuronal activity [3]. We have previously argued [17] that metabolite levels may reflect the decrease in nigro-striatal neuronal activity consequent to AMPH administration. Although it is generally proposed that AMPH-induced release of DA is dependent on neuronal activity, higher doses of AMPH can still release some DA in the absence of neuronal activity [5]. Coupled with AMPH blockade of DA reuptake, this release would presumably maintain synaptic levels of DA in the absence of dopaminergic neuronal firing. One might then speculate that the inhibition of nigro-striatal dopaminergic neurons by AMPH and the consequent dissociation of dopaminergic transmission from functioning DA neuronal activity may play a permissive role in the appearance of focused stereotyped behavior.

It is not possible from the data presented above to specify whether the enhanced decline in striatal metabolite levels following chronic AMPH reflects altered sensitivity of the

DA neuron to the drug or reflects a change in some system which modulates the activity of the nigro-striatal DA pathway. Two potential mechanisms for the enhanced responsiveness of these neurons warrant some attention. Kuhn and Schanberg [18] reported that chronic AMPH caused an increase in brain levels of AMPH at early times after challenge with the drug, relative to chronic saline treated controls. Increased levels of AMPH would be expected to exert greater effects on CNS DA metabolism. However, the increase was observed only after a chronic dosage regimen consisting of increasing AMPH doses from 10 to 30 mg/kg twice daily for 10 days, and not after 5 mg/kg twice daily for 10 days. In addition, increased AMPH levels were found throughout the brain (with the exception of the cerebellum) whereas the enhanced decline in DOPAC and HVA described above occurs in striatal but not mesolimbic tissue. Thus, changes in AMPH metabolism which they reported do not appear relevant to the behavioral changes of interest.

Alternatively, Muller and Seeman [24] reported a 19% decrease in ^{3}H-haloperidol binding after chronic AMPH. Those authors interpreted their data as reflecting a decrease in autoreceptor number which may modulate the release of DA. The decrease in autoreceptors would lead to an increase in synaptic DA levels and could be the basis for sensitization to AMPH. Increased striatal synaptic DA levels would be expected to trigger striato-nigral inhibitory feedback at lower doses of AMPH, more readily inhibiting nigro-striatal DA neuronal activity and decreasing levels of DOPAC and HVA. However, Staunton *et al.* [33] found no such change in the threshold dose of AMPH needed to inhibit the firing of nigral DA neurons following a behaviorally relevant regimen of chronic AMPH.

Thus the more rapid decline in striatal DOPAC and HVA levels after chronic AMPH pretreatment may account for the more rapid onset of the stereotyped response after challenge with AMPH, as well as the appearance of focused stereotypy at doses of the drug which would acutely not produce this behavioral response. The data presented in Fig. 1, however, indicate that AMPH-induced changes in DA function during the chronic pretreatment phase are necessary for the subsequent expression of *all* phases of the reverse tolerance phenomenon. The data at the early time points is somewhat equivocal in that, whereas the more rapid onset of stereotypy does not occur in animals pretreated with both HAL and AMPH, neither are these animals identical to the saline pretreated group. Thus it is possible that failure to observe the more rapid onset of stereotypy may reflect the superposition of a behavioral response more specific to the HAL pretreatment. However, the co-administration of low doses of HAL with AMPH during the chronic AMPH administration clearly blocked the apparent increased intensity of the stereotyped response and the enhanced post-stereotypy locomotor activity—behavioral changes for which no corresponding changes in dopaminergic function were detected. Similarly the tolerance which develops with chronic AMPH administration to AMPH effects on self-stimulation behavior and in discriminative stimulus studies can be blocked by the co-administration of HAL during the chronic regimen [2]. Again, no changes were detected in the dopaminergic biochemical response to AMPH after the 3X daily chronic regimen which were specific to that regimen. Although the half-life of HAL is sufficiently long to result in accumulation following chronic administration [25], the fact that chronic HAL treatment did not change the acute response to AMPH with respect to these behaviors indicates that any accumulation of HAL that may have occurred was not functionally significant in these studies.

It would appear that while an interaction of AMPH with functional DA transmission is essential for the appearance of the behavioral phenomena of tolerance and reverse tolerance after chronic AMPH, the molecular changes which subserve these behavioral changes are not likely localized to striatal or mesolimbic DA neurons, except in the case of the more rapid onset of stereotypy. The idea that this change in stereotypy and the enhanced post-stereotypy locomotor activity which occur as a consequence of chronic AMPH may be subserved by more than one distinct mechanism is consistent with the recent observation (Leith and Kuczenski, manuscript in preparation) that the development of these two characteristics during chronic AMPH may be separated in different strains of rats. While all rats develop the more rapid onset of stereotypy, some strains do not develop the post-stereotypy hyperactivity. Although, the data presented here do not rule out the possibility that another dopaminergic system may provide the neural substrate for the behavioral changes induced by chronic AMPH, the apparent involvement of striatal and mesolimbic DA systems in AMPH-induced locomotor activity and stereotypy following acute administration suggests that these loci provide likely regions for such changes to occur. Because these changes depend on mechanisms which can be blocked by HAL, the data suggest that DA neurons are a critical link in the chain of events that occurs during chronic AMPH administration, but that the actual changes are occurring at sites on which DA neurons impinge and in ways that are not reflected by compensatory changes in presynaptic dynamics.

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