THE LONG-TERM EFFECTS OF REPEATED AMPHETAMINE TREATMENT
IN VIVO ON AMPHETAMINE, KCl AND ELECTRICAL STIMULATION
EVOKED STRIATAL DOPAMINE RELEASE *IN VITRO*

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Summary

Exposure to amphetamine (AMPH) *in vivo* produces an enduring enhancement ('sensitization') in AMPH-stimulated striatal DA release *in vitro*. Experiments were conducted to determine whether striatal DA release evoked by neuronal depolarization is altered by AMPH pretreatment in a similar manner. It was found that AMPH pretreatment produced a long-lasting (at least one week) enhancement in striatal DA release evoked by AMPH, KCl or electrical field stimulation. In contrast, the basal rate of DA efflux was not altered by pretreatment condition. A mechanism by which a single change in the intracellular distribution of DA could enhance both AMPH- and depolarization-induced DA release is proposed.

In humans chronic amphetamine (AMPH) abuse frequently results in the development of a drug-induced psychosis (AMPH psychosis) that is clinically similar to paranoid schizophrenia (1-4). In nonhuman animals repeated treatment with AMPH produces a progressive enhancement in the motor stimulant effects of AMPH (behavioral sensitization), and this phenomenon is considered an animal analogue of AMPH psychosis (5, 6). There has been considerable interest, therefore, in the neurobiological basis of behavioral sensitization. Research on this question has focused on mesotelencephalic dopamine (DA) systems, in part because AMPH is thought to produce many of its effects on behavior by enhancing dopaminergic activity, especially DA release (7-9).

Many neural correlates of behavioral sensitization have been reported in the literature (5), and of particular relevance here are reports that behavioral sensitization is accompanied by an enhancement in AMPH-stimulated striatal DA release *in vitro* (10-12) This change in AMPH-stimulated DA release can account for a number of features of the behavioral phenomenon. For example, both behavioral sensitization and an increase in AMPH-stimulated striatal DA release *in vitro*: (a) can be produced by a single injection of AMPH (12, cf.13); (b) persist for a very long time following the cessation of drug treatment (10,11); and (c) are similarly influenced by how long animals are withdrawn from AMPH pretreatment (10). It has been suggested, therefore, that an enduring change in the releasability of DA may be responsible for some of the enduring changes in behavior produced by past experience with AMPH (5, 11).

To narrow down the mechanism(s) by which prior AMPH treatment enhances striatal DA release it is important to determine if the effect is specific to AMPH-stimulated DA release, or whether the DA

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release produced by neuronal depolarization is changed in a similar manner This is because DA release stimulated by AMPH is due to a different process than the neurotransmitter release associated with impulse flow and the depolarization of axonal terminals (9) Depolarization-induced DA release is thought to occur by Ca^{++}-dependent exocytosis, whereas AMPH-stimulated DA release is thought to occur by a Ca^{++}-independent exchange-diffusion process involving the DA reuptake carrier (9, 14) To address this question experiments were conducted using an *in vitro* superfusion technique to determine the effects of AMPH pretreatment on endogenous striatal DA release elicited by either (a) AMPH, (b) KCl depolarization; or (c) depolarization by electrical field stimulation.

Methods

Subjects and Surgical Preparation

Adult female Holtzman rats (Holtzman Co., Madison, WI) were housed individually with food and water freely available. The colony room was temperature regulated and lights were maintained on a 14:10 hr light.dark cycle (lights on at 08:00 hr). Seven to 14 days before the start of an experiment animals were ovariectomized under ether anesthesia Ovariectomized female rats were used for the following reasons: (a) female rats show more robust behavioral sensitization than do males (15, 16); and (b) ovariectomy eliminates the variation in striatal DA release associated with hormonal fluctuations across the estrous cycle (17), but has no effect on sensitization (16, 18)

Quantification of Behavior

After each pretreatment injection of AMPH or saline animals were immediately replaced into their home cage and behavior quantified by direct visual observation and rating of stereotyped behavior. Animals were rated during a 1 min observation period every 20 min following the injection, for a total of 3 hr. Overall stereotyped behavior was rated with a scale adapted from MacLennan and Maier (19). Individual components of stereotyped behavior (i e., sniffing, repetitive head and limb movements and oral behaviors) were rated as described by Rebec and Segal (20). A daily cumulative score for each rating scale was calculated by summing the ratings obtained during each test session. In addition, the total volume of water ingested over 5 hr following each injection was recorded.

In Vitro DA Release

One week after the last pretreatment with AMPH each rat was killed by decapitation, and the brain was quickly removed and placed into ice-cold medium Coronal sections were obtained using a cutting block similar to that described by Heffner, Hartman and Seiden (21), and after each striatum was chopped into 1 mm^3 pieces it was placed into a superfusion chamber. The superfusion chambers and methods have been described previously (22). Briefly, the superfusion medium consisted of a modified Krebs-Ringer phosphate buffer with a final composition of: 120 mM NaCl, 4.8 mM KCl, 1.25 mM $CaCl_2 \cdot 2H_2O$, 1 5 mM $MgSO_4$, 15 mM phosphoric acid, 0.1% bovine serum albumin, 10 mM glucose and pH 7.4 The medium was oxygenated with 95% $O_2 \cdot 5\% CO_2$ for 15 min and stirred continuously throughout the experiment Superfusion chambers were maintained at 34°C, and medium was pumped through the chambers at 100 µl/min After tissue was placed into a superfusion chamber it was left to equilibrate for 65 min before beginning sample collection. Samples were then collected over 5-min intervals in ice-chilled tubes containing 25 µl of 0.5 N $HClO_4$ with dihydroxybenzylamine added as an internal standard. Samples were stored at -20° C until assayed by high performance liquid chromatography with electrochemical detection, as described previously (22)

The data from each superfusion were examined by an experienced judge (J B.B.) who was blind to the treatment conditions, and chambers that failed to meet the following criteria were not considered viable and were excluded from the experiment For a chamber to be considered viable (a) stimulated DA release had to be greater than basal DA efflux (i.e., there was not a progressive decrement in DA efflux); and (b) at the end of the experiment the tissue had to respond to a 60 mM KCl challenge by an increase in DA release (indicating that the tissue was still viable) Chambers

were usually excluded for reasons directly attributable to experimenter error or equipment malfunction, and there was no group bias in the number of chambers excluded. These methods were used in all of the following experiments, and procedures specific to each experiment are described below.

Experiment 1: The effect of AMPH pretreatment on AMPH-stimulated striatal DA release in vitro: dose-response relations

Amphetamine pretreatment Animals were either pretreated with 3.0 mg/kg of *d*-AMPH sulfate (i.p.) dissolved in 0.9% saline (weight of the salt) once daily for 6 consecutive days, or left undisturbed in their home cage (non-handled). Non-handled controls were used in this experiment to minimize the stress-induced sensitization of dopaminergic activity sometimes produced by repeated saline injections (23, 24).

Superfusion After the 65 min equilibration period (see above) 3 baseline samples were collected over 15 min. At the beginning of the next interval medium containing *d*-AMPH was infused for either 5 min (with 0.5, 2.75 and 5.0 μM AMPH), or 2.5 min (with 10 μM AMPH), and samples collected for an additional 30 min (all independent groups). Finally, 60 mM KCl was infused for 2 5 min and one additional 5-min sample was collected. It should be noted that the doses of AMPH used here are just at threshold for evoking consistent DA release in this apparatus, representing the extreme far left and nonlinear portion of the dose-response curve for AMPH-evoked striatal DA release (see Fig. 4 in ref. 22). It was hoped that this would maximize the probability of detecting an increase in the releasability of DA due to pretreatment condition.

Experiment 2: The effect of AMPH pretreatment on KCl-evoked DA release

Amphetamine pretreatment. Animals received 3 0 mg/kg of *d*-AMPH or 1.0 ml/kg of 0.9% saline once a week for 5 weeks (a total of 5 injections).

Superfusion One week after the last injection of AMPH or saline striatal tissue was placed into superfusion chambers as described above, except after baseline the tissue was stimulated by including 25, 35 or 45 mM KCl in the medium for 5 min (the concentration of NaCl was reduced proportionately). The DA release evoked by these concentrations of KCl has been previously shown to be Ca++ and temperature-dependent (22).

Electrical stimulation-induced DA release

Apparatus The top and bottom of each superfusion chamber was fitted with a Ag-AgCl electrode (In Vivo Metric Systems, Healdsburg, CA). The circular surface of the top electrode had a cross-sectional diameter of 1 5 mm and the bottom electrode a diameter of 4 0 mm. The electrodes were soldered with Ag solder to lead wires and the solder joint reinforced with a conductive Ag epoxy adhesive (Tra-Con). The electrodes were insulated with teflon heat-shrink tubing, except for the cross-sectional area of the tip, and were separated by 12 mm (which maintained a 200 μl volume in the chambers) Nylon mesh spacers were used to prevent tissue from resting directly on the electrode surface

Ag-AgCl electrodes were used rather than platinum, which is frequently used for *in vitro* stimulation experiments, for several reasons: (a) It has been reported that platinum acts as a catalyst for the oxidation of neurotransmitter substrates or of glucose, but Ag has minimal deleterious effects on tissue viability compared to other metals (25-27), (b) Ag apparently does not affect tissue metabolism (25), and (c) chlorided electrodes are maximally inert to physiological solutions and resistant to electrolytic processes (25, 27)

The electrical stimulus was provided by a Grass S8 stimulator and consisted of two rectangular D.C pulses that were passed through individual stimulus isolation units (Grass Model SIU 478A) and individual constant current units (Grass Model CCU1A). The second pulse was delayed by the duration of the first pulse and its polarity reversed at the constant current unit to create a true biphasic pulse. Biphasic pulses were used to minimize the tissue damage and electrode polarization

sometimes associated with the use of monophasic pulses *in vitro* (28). Current was monitored by recording the voltage drop across a resistor located in series with the chamber.

Pilot studies. A series of pilot experiments were conducted to validate the electrical stimulation procedures and to determine suitable stimulus parameters. First, the intensity, pulse duration and pulse frequency were varied to determine parameters that evoked a consistent enhancement in DA release. Second, the effect of varying the stimulus frequency (5, 10, 20, 30, 40, or 50 pulse/sec, [pps]) on DA release was determined, while current (40 mA) and pulse duration (2 msec) were held constant.

The third issue addressed in pilot studies was whether the release produced by a highly effective stimulus was sensitive to calcium and temperature. With electrical stimulation it is possible to produce nonselective neurotransmitter release with high intensity stimulation (29) It is difficult, however, to compare stimulation parameters from lab-to-lab because resistance varies greatly with such things as chamber volume, the density of tissue packing, and the surface area and distance between the electrodes. As pointed out by Orrego (28, p 1048) it is not possible to know the actual stimulating current because, "most of the current that flows in the system is short circuited through the bulk of the liquid" Therefore, superfusion experiments were conducted as described above, except chambers were tested with either: (a) normal medium at 34°C, (b) medium without additional Ca++ (magnesium was increased to 2.45 mM) and at 34°C, or (c) normal medium, but at 0°C Stimulus parameters that produced a rate of DA release of approximately 200 pg DA/mg tissue/min were used, which consisted of 40 mA, 2 msec pulses applied at 50 pps for a total of 2 min.

Experiment 3: The effect of AMPH pretreatment on electrical stimulation-evoked DA release

Amphetamine pretreatment. Animals received either an i p injection of 3.0 mg/kg of *d*-AMPH sulfate or 1 ml/kg of 0 9% saline once a day for 6 consecutive days An additional group was left undisturbed in their home cages during this time (nonhandled). Behavior was monitored only following the first and sixth injection, as described above

Superfusion One week after the last injection superfusion experiments were conducted as previously described, except 20-min fractions were collected for a total of 3 samples After one baseline sample was collected the tissue was stimulated with electrical field stimulation consisting of biphasic rectangular pulses (20 mA, 2 msec duration) applied for 10 sec on/10 sec off for 40 min Independent groups of chambers were stimulated at either 5, 10, or 20 pps It should be noted that the highest rate of stimulation used in this experiment (20 pps) was ten times lower than used in the pilot experiment to determine Ca++ and temperature dependence (0 8 mCoulombs/sec *vs* 8 mCoulombs/sec, respectively), and that each chamber was tested with only one frequency.

Results

Behavior.

All of the AMPH pretreatment regimens produced a similar and significant enhancement in the motor stimulant effects of AMPH (i e , behavioral sensitization) Data are shown only for Exp. 2 to illustrate typical behavioral effects (also see 15, 18, 30). Fig 1 shows that repeated AMPH treatment produced a progressive and significant enhancement in the ratings of overall stereotypy, sniffing and repetitive head movements, and in the amount of water ingested There was no effect of repeated AMPH treatment on oral stereotypies (data not shown), consistent with previous reports (20, 31)

Experiment 1: The effect of AMPH pretreatment on AMPH-stimulated striatal DA release in vitro.

Fig 2 shows that the inclusion of AMPH in the superfusion medium enhanced endogenous striatal DA release above basal levels of DA efflux in all groups (i e , all means are significantly greater than 0). There was no effect of AMPH pretreatment on AMPH-stimulated DA release evoked by infusion of 0 5 µM or 2 75 µM AMPH for 5 min However, infusion of 5 µM AMPH

for 5 min, or 10 µM for 2.5 min, produced a significantly greater increase in DA release in the AMPH pretreated group than in the control group (Fig. 2).
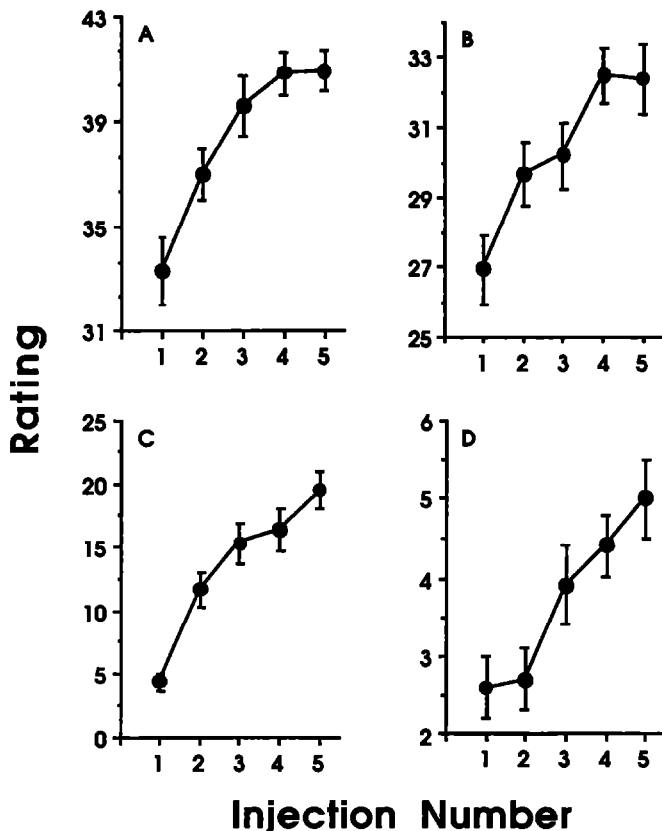

Fig. 1. The effect of repeated AMPH treatment on stereotypy ratings and drinking behavior. The symbols represent the mean (±S.E.M ; N = 38) values after each of 5 injections of 3 0 mg/kg of AMPH given at weekly intervals There was a significant increase in the ratings of overall stereotypy (A), sniffing (B), and head and limb movments (C; comparison of first and last injection, Wilcoxon tests, p's < 0 001) The increase in water consumption (ml) was also significant (D, paired t-test, p < 0 001)
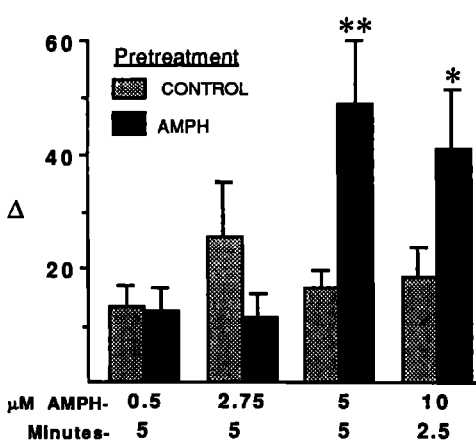
Fig. 2. The effect of pretreatment with AMPH on AMPH-stimulated endogenous striatal DA release in vitro. The bars represent the mean (+S.E.M.) change (Δ) in DA efflux from baseline, expressed in pg DA/mg tissue/min (i e., a value of zero represents no change from baseline) The groups did not differ in the basal rate of DA efflux, which averaged 23.97±0.97 pg/mg/min across all groups. There was no effect of pretreatment condition on DA release evoked by either 0 5 or 2.75 µM AMPH. However, there was a greater increase in DA efflux from the AMPH-pretreated group when the tissue was challenged with 5 µM of AMPH for 5 min or 10 µM for 2.5 min (planned 1-tailed t-tests, t[8]=3 15, **p=0 007, t[7]=2.22, *p=0 031, respectively)

Experiment 2: The effect of AMPH pretreatment on KCl-evoked striatal DA release.

Fig. 3 shows that the addition of 25 to 45 mM KCl to the superfusion medium increased endogenous DA release above the basal level of DA efflux in all groups (i.e., all means are greater than 0). More importantly, KCl produced a greater increase in DA release in the AMPH-pretreated group than in the control group. The 2-way analysis of variance resulted in a significant main effect and no significant interaction, but inspection of Fig. 3 reveals a tendency for a larger effect of pretreatment condition with the higher concentrations of KCl
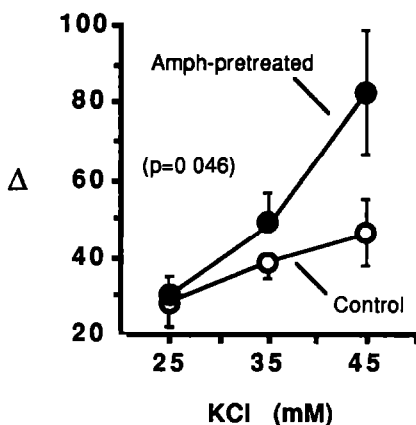
Fig. 3 The effect of pretreatment with AMPH (solid circles) on endogenous striatal DA release evoked by 25, 35 or 45 mM KCl in vitro The symbols represent the mean ($\pm S$ E M) change (Δ) in DA efflux from baseline, expressed in pg DA/mg tissue/min. There were no group differences in the basal rate of DA efflux, which averaged $18\ 22\pm1\ 71$ pg/mg/min across all groups However, a 2-way analysis of variance on the change in DA efflux produced by KCl resulted in a significant effect of pretreatment condition ($F[1, 32] = 4\ 17$, $p = 0\ 046$), a significant effect of KCl concentration ($F[2, 32] = 6\ 99$, $p = 0\ 003$) but no significant interaction

**Electrical stimulation-induced DA release: Pilot experiments.**

A 40 mA, 2 msec stimulus applied for 2 min did not produce a significant increase in DA efflux at frequencies below 20 pps. As the stimulus frequency was increased above 20 pps DA efflux increased to an average of approximately 200 pg DA/mg tissue/min at 50 pps. The increase in DA efflux produced by the highest intensity stimulus tested (40 mA, 2 msec, 50 pps) was reduced by 74% if Ca^{++} was excluded from the medium, and this stimulus was completely ineffective in inducing DA release when the chambers were kept at 0°C

Experiment 3: The effect of AMPH pretreatment on electrical stimulation-evoked DA release.

Electrical stimulation significantly enhanced DA release above the basal levels of DA efflux in all groups (i e., all means are greater than 0; Fig 4) There was no difference between the saline-pretreated and nonhandled control groups, and therefore they were pooled for comparison with the AMPH-pretreated group. Electrical stimulation evoked a greater change in DA release in the AMPH-pretreated group than in the control group, as indicated by a significant main effect of pretreatment condition (2-way ANOVA) The ANOVA did not result in a significant interaction, but inspection of Fig. 4 reveals a tendency for a greater effect at the highest stimulus frequency

Discussion

The AMPH pretreatment regimens used in the present experiments produced behavioral sensitization, as expected from previous studies (30, 32-34, 5 for review). Furthermore, behavioral sensitization was accompanied by a dose-related enhancement in AMPH-stimulated striatal DA release in vitro, which confirms and extends previous studies (10-12). Most importantly, AMPH pretreatment also produced an enduring enhancement in the Ca^{++}-dependent release of DA evoked by high KCl or electrical field stimulation. These latter studies establish that sensitization-related changes in striatal DA release are not unique to AMPH-stimulated DA release
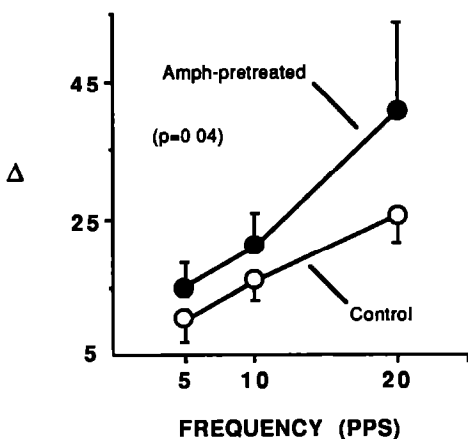
Fig. 4. The effect of pretreatment with AMPH (solid symbols) on endogenous striatal DA release in vitro evoked by electrical field stimulation applied at 5, 10 or 20 pulses/sec (PPS). The symbols represent the mean (±S.E.M.) change (Δ) in DA efflux from baseline, expressed in pg DA/mg tissue/min. There were no group differences in the basal rate of DA efflux, which averaged 16.53±1.06 pg/mg/min across all groups. However, a 2-way analysis of variance on the change in DA efflux produced by electrical stimulation resulted in a significant effect of pretreatment condition $(F[1, 82] = 4.24, p = 0.04)$, a significant effect of stimulus frequency $(F[2, 82] = 8.95, p < 0.001)$ but no significant interaction

However, not all challenge doses of AMPH were effective. Striatal tissue from AMPH pretreated animals showed a greater elevation in DA release than tissue from control animals when challenged with 5 μM AMPH for 5 min or 10 μM for 2.5 min, but not when challenged with lower doses (0.5 and 2 75 μM). In comparison, Robinson & Becker (11) reported sensitization-related changes in striatal DA release when 1 μM of AMPH was infused continuously for 10 min. Similarly, using an incubation procedure, Kolta et al. (10) found that both 1 μM and 10 μM AMPH were effective when applied for 15 min, although the 10 μM challenge more clearly differentiated the AMPH pretreated and control groups than did the 1 μM challenge. In one study, a much higher dose of AMPH (1 mM) was delivered as a brief pulse, which quickly washed out of the chamber, and this also resulted in a sensitization-related enhancement in striatal DA release (12). Taken together, the available evidence suggests that the ability of an *in vitro* AMPH challenge to reveal sensitization-related changes in striatal DA release is influenced by the duration and concentration of the AMPH challenge. The effects of AMPH pretreatment on AMPH-stimulated DA release may be seen under some experimental conditions, but not others

The effect of AMPH pretreatment on the Ca^{++}-dependent DA release evoked by either high KCl or electrical field stimulation reported here appears to be discrepant with a previous study from this laboratory, in which it was found that KCl-evoked striatal DA release was not enhanced in sensitized rats (11). The most probable explanation for this is an order effect. In the Robinson and Becker (11) study striatal tissue was always exposed first to AMPH, and then to KCl. Therefore, AMPH may have depleted the amount of DA available for subsequent KCl-stimulated DA release It is thought, for example, that AMPH displaces bound DA from storage pools (14), which would leave less DA available for subsequent release by KCl. There was no such order effect in the present experiments, because the data are based only on the initial response to either AMPH, KCl or electrical stimulation.

As mentioned in the introduction, the process thought to mediate AMPH-stimulated DA release (Ca^{++}-independent, carrier-mediated exchange-diffusion) is very different than that thought to mediate DA release produced by depolarization of axonal terminals (Ca^{++}-dependent exocytosis). Therefore, the observation that sensitization is accompanied by an enhancement of not only AMPH-stimulated DA release, but also of KCl and electrical stimulation-evoked DA release, has important implications for hypotheses regarding the neurobiological basis of the enhancement in DA release. Either the sensitization-related enhancement in DA release is due to more than one mechanism, for example, one responsible for the enhancement in AMPH-stimulated DA release and another for the enhancement in depolarization-induced DA release, or, a mechanism common to both AMPH and depolarization-induced DA release is involved.

There are many neuronal adaptations that could lead to increased DA release, but most have difficulty accounting for changes in both depolarization-induced DA release and AMPH-stimulated DA release For example, it has been suggested that the sensitization-related enhancement in DA

release and AMPH-induced behavior may be due to a subsensitivity of DA autoreceptors (35-37).
But AMPH-stimulated DA release does not seem to be modulated by DA autoreceptors (38), and
therefore, it is not clear how a change in DA autoreceptors could account for the sensitization-related
enhancement in AMPH-stimulated DA release and AMPH-evoked behavior. Similarly, a role for
Ca++ in AMPH sensitization is suggested by a report of increased striatal calmodulin levels in AMPH
pretreated rats (39) But AMPH-stimulated DA release is not Ca++-dependent, and therefore, it is
not obvious how changes in Ca++ influx or a Ca++-binding protein could account for changes in
AMPH-stimulated DA release and AMPH-elicited behavior A change in AMPH-stimulated DA
release could be due to an alteration in the reuptake carrier. But the Ca++-dependent release produced
by KCl is not affected by the presence of the DA reuptake blocker nomifensine, and this suggests
that depolarization-induced release occurs independently of the uptake mechanism (40). Therefore,
changes in the uptake mechanism could mediate the enhancement in AMPH-stimulated release, but
presumably not the effects of AMPH pretreatment on Ca++-dependent DA release

The idea that a common mechanism mediates the enhancement in both AMPH-stimulated and
depolarization-induced striatal DA release should be given serious consideration, not only because it
is more parsimonious, but because it could also help explain the interchangeability of AMPH and
stress in producing sensitization. Animals sensitized to AMPH exhibit enhanced behavioral and
neurochemical responses to subsequent stress (23, 41, 42), and prior stress enhances the behavioral
and neurochemical responses to a subsequent AMPH challenge (23, 24, 41, 43-45).

How could a single change alter the releasability of DA to both AMPH and depolarization? It
is not due to just an increase in the total amount of presynaptic DA, because AMPH pretreatment
does not alter mesotelencephalic DA concentrations (15, 18, 46-48, 5 for review). But it is
important to consider that DA is probably located in at least 3 different compartments, or 'pools',
and release occurs more readily from some pools than from others (9, 49-52). A free cytoplasmic
pool is thought to contain newly synthesized DA, largely because tyrosine hydroxylase and dopa-
decarboxylase are not associated with synaptic vesicles (52) AMPH seems to release DA selectively
from this pool. Metabolically older neurotransmitter is presumably bound within storage
compartments, for protection from metabolism, until it is released by a Ca++-dependent release
process. Bound pools of DA might correspond to vesicular stores, and there are probably two pools
of bound DA; one that is readily releasable by the arrival of an action potential at the nerve terminal
('releasable bound DA') and another that is not ('inactive bound DA') The difference between these
two bound pools may be that releasable bound DA is simply closer to the neuronal membrane than
the inactive pool, and thus has greater access to release sites (50) A redistribution of DA among
these pools, with no change in total DA, could lead to an increase in both AMPH and depolarization-
induced DA release in the following way

A transfer of DA from the inactive bound pool into the releasable bound pool would obviously
result in more DA readily available for release upon depolarization of the terminal. There would be
no alteration in the size of the cytoplasmic pool in the absence of an AMPH challenge After AMPH
is taken up into a DA terminal, however, it not only induces DA release into the extracellular space
by exchange-diffusion, but it also displaces bound DA into the cytoplasm (9, 14). Thus, if AMPH
pretreatment enhanced the size of the releasable bound pool, for example, by a physical translocation
of vesicles closer to release sites on the presynaptic membrane, then presumably they would also be
closer to reuptake sites on the presynaptic membrane. AMPH that had just entered the cytoplasm
would, therefore, have access to an augmented releasable bound pool and a greater opportunity to
displace DA from this augmented releasable bound pool This would increase the amount of DA in
the cytoplasmic pool, resulting in more DA being readily available for exchange-diffusion as AMPH
entered the cell. In conclusion, it is suggested that a single change - an increase in the size of the
releasable bound pool of DA - could result in an enhancement of both AMPH and depolarization-
induced DA release

This hypothesis is speculative, and further research is required to determine the neurobiological
basis of the enhancement in DA release produced by AMPH pretreatment Nevertheless, the present
study suggests that exploration of presynaptic mechanisms that concomitantly regulate both uptake
carrier-mediated chemical release and impulse-related Ca++-dependent release may provide a

promising strategy for increasing our understanding of the biological basis of behavioral sensitization

In summary, it was shown that the behavioral sensitization produced by repeated intermittent injections of AMPH is accompanied by a persistent enhancement in AMPH-stimulated endogenous striatal DA release, and in Ca++-dependent DA release evoked by high KCl or electrical stimulation *in vitro*. These results support the hypothesis that an enhancement in striatal DA release may be at least partly responsible for the behavioral sensitization seen in AMPH-pretreated animals (5, 11), and perhaps even the hypersensitivity to the psychotogenic effects of AMPH seen in former AMPH addicts (53-55). Of course, this does not exclude the possibility that changes in other neural systems are also involved (eg. 5, 23, 41, 45, 56). The observation that sensitization-related changes in DA release are not unique to AMPH-stimulated DA release is also consistent with the hypothesis that behavioral sensitization is not unique to the psychopharmacology of AMPH (56).

Acknowledgements

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