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d-AMPHETAMINE AND γ-BUTYROLACTONE ALTERATION OF DOPAMINE SYNTHESIS IN THE TERMINALS OF NIGROSTRIATAL AND MESOLIMBIC NEURONS

POSSIBLE ROLE OF VARIOUS AUTORECEPTOR SENSITIVITIES

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Abstract—The rate of synthesis of dopamine (DA) was estimated *in vivo* in terminals of nigrostriatal and mesolimbic neurons by measuring the accumulation of dihydroxyphenylalanine (DOPA) in the striatum and in the nucleus accumbens and olfactory tubercle of male rats following the administration of an inhibitor of aromatic L-amino acid decarboxylase. Low to moderate doses of *d*-amphetamine (0.5 to 1.0 mg/kg) increased DA synthesis in the striatum but were without effect in the nucleus accumbens and olfactory tubercle; a higher dose (5.0 mg/kg) did not alter DA synthesis in any of the brain regions. Despite the lack of effect of *d*-amphetamine *per se* in the nucleus accumbens and olfactory tubercle, in both of these regions, as well as in the striatum, this drug was effective in reversing the decreased DA synthesis induced by a monoamine oxidase inhibitor (nialamide) or a DA agonist (apomorphine). γ-Butyrolactone (GBL) increased DA synthesis in the striatum, nucleus accumbens and olfactory tubercle, but it was less potent, less effective, and had a shorter duration of action in the latter two regions. *d*-Amphetamine, like GBL, may exert part of its effect on DA synthesis by reducing impulse flow in ascending DA neurons, since a low dose of *d*-amphetamine (0.125 mg/kg) potentiated the GBL-induced increase in DA synthesis in striatum, nucleus accumbens and olfactory tubercle. Regional differences in the actions of GBL and *d*-amphetamine on DA synthesis may be related to differences in receptor-mediated mechanisms that regulate the synthesis of this amine in neurons terminating in the striatum versus those terminating in nucleus accumbens and olfactory tubercle. At least one difference appears to be in the sensitivity of DA autoreceptors in these regions. The dose of apomorphine needed to reverse the GLB-induced increase of DA synthesis in nucleus accumbens and olfactory tubercle was approximately 1/10 that necessary to cause the same effect in the striatum. Pretreatment with a DA antagonist (haloperidol) potentiated the GBL-induced stimulation of DA synthesis in nucleus accumbens and olfactory tubercle, but not in the striatum. These results suggest that the reduced abilities of GBL and amphetamine to increase DA synthesis in nucleus accumbens and olfactory tubercle may result from a greater sensitivity of autoreceptors on mesolimbic DA neurons permitting a tighter regulatory control over DA synthesis in these neurons.

The regulation of dopamine (DA) synthesis has been examined primarily in the striatum, which contains terminals of nigrostriatal DA neurons (see reviews in Refs. 1 and 2). Less attention has been paid to mesolimbic DA neurons that project from the ventromedial tegmentum to limbic forebrain regions (e.g. nucleus accumbens and olfactory tubercle [3]). The synthesis of DA in both nigrostriatal and mesolimbic neurons is believed to be regulated by end-product feedback inhibition [4], by neuronal feedback loops and alterations in neuronal impulse flow [5, 6], and by autoreceptor activation [2]. There are quantitative, but not major qualitative, differences in the mechanisms which regulate DA synthesis in nigrostriatal and mesolimbic DA neurons [7, 8].

The nigrostriatal and mesolimbic DA neuronal systems have been implicated in different components of the behavioral response to *d*-amphetamine [9, 10]. Stereotyped behaviors produced by *d*-amphetamine in rats are abolished by the destruction of DA terminals in the striatum but not in the nucleus accumbens. Conversely, amphetamine-induced locomotor activity is blocked by the destruction of DA terminals in nucleus accumbens but not in the striatum. These observations, coupled with the fact that different behavioral effects of *d*-amphetamine are dose dependent, with activation of locomotor activity at low doses and stereotyped behavior occurring at higher doses, suggest differences in the mechanisms by which this drug influences nigrostriatal and mesolimbic DA neuronal systems [11].

d-Amphetamine has a differential action on the *in vivo* rate of DA synthesis in terminals of nigrostriatal and mesolimbic neurons; it increases the rate of dihydroxyphenylalanine (DOPA) accumulation after the administration of an inhibitor of aromatic L-amino acid decarboxylase in the striatum, but not in other brain regions [12–15]. This suggests that the

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mechanisms which regulate DA synthesis in various neuronal systems are different. The present studies were designed to examine the mechanism(s) by which the synthesis of DA is regulated in the terminals of nigrostriatal and mesolimbic DA neurons. Two drugs, *d*-amphetamine and γ-butyrolactone (GBL), were used as tools in these studies.

d-Amphetamine has complex actions on DA neurons; it releases DA from nerve terminals [16, 17], and it blocks the reuptake of DA into the presynaptic nerve terminal [18, 19]. The result of these actions is to facilitate DA transmission. On the other hand, *d*-amphetamine causes a compensatory reduction in the firing of nigrostriatal [20] and mesolimbic [21] DA neurons. GBL also reduces impulse traffic in both nigrostriatal and mesolimbic DA neurons [22] but, unlike *d*-amphetamine, this drug does not release or block the reuptake of DA at the nerve terminal. In the present studies, the actions of *d*-amphetamine and GBL were compared on the *in vivo* rate of DA synthesis in striatum, nucleus accumbens, and olfactory tubercle of rats. Preliminary results of these studies have been presented [23].

EXPERIMENTAL PROCEDURES

Male Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, MI), weighing 225–300 g, were maintained under 12 hr periods of light and dark. Food (Wayne Lablox) and water were available *ad lib*. *d*-Amphetamine SO_4, apomorphine HCl (Eli Lilly & Co., Indianapolis, IN), nialamide and 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015; Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% saline. Doses refer to the respective salts of these drugs. Haloperidol (McNeil Laboratories, Fort Washington, PA) was dissolved in 0.3% tartaric acid. γ-Butyrolactone (Matheson, Coleman & Bell, Norwood, OH) was diluted with saline before use.

The *in vivo* rate of DA synthesis was estimated in various brain regions by measuring accumulation of DOPA after the administration of NSD 1015 (100 mg/kg); this inhibitor of aromatic L-amino acid decarboxylase was injected i.p. 30 min prior to decapitation. The brain was removed, and the striatum, olfactory tubercle and nucleus accumbens were dissected as previously described [24]. The tissues were homogenized in various volumes of cold 0.2 N perchloric acid containing 10 mg/100 ml ethyleneglycolbis(amino-ethylether)tetra-acetate (EGTA) such that approximately 10 mg tissue was homogenized in 100 μl. The homogenate was centrifuged in a Beckman microfuge, and the protein content of the pellet was determined as previously described [25]. The DOPA content was determined in 10-μl aliquots of the supernatant fraction using a radioenzymatic assay [26]. In the absence of a decarboxylase inhibitor, the concentration of DOPA in the brain regions is essentially zero (not significantly different from the reagent blank), but following the administration of 100 mg/kg NSD 1015 the concentration of DOPA increases linearly with time for at least 30 min [26, 27]. The accumulation of DOPA after the administration of a decarboxylase inhibitor has been used previously as an *in vivo* estimate of the rate of DA synthesis in the striatum and mesolimbic regions [2, 7, 12, 15, 28].

Differences between treatment groups were initially evaluated by a one-way analysis of variance; differences between treatment means were then determined using the Student–Newman–Keuls' test [29].

RESULTS

Effect of d-*amphetamine on* in vivo *DA synthesis in terminals of nigrostriatal and mesolimbic neurons.* The accumulation of DOPA was increased markedly in the striatum following the administration of 0.5 to 1.0 mg/kg *d*-amphetamine while after 5 mg/kg the rate of DA synthesis was not different from control (Fig. 1); a similar biphasic response to *d*-amphetamine has been reported previously [30]. On the other hand, *d*-amphetamine had no effect on the rate of accumulation of DOPA in the nucleus accumbens and olfactory tubercle. The relatively selective action of *d*-amphetamine on DA synthesis in the striatum confirms previous reports [12–15].

Effect of d-*amphetamine on the nialamide- and apomorphine-induced decrease in DA synthesis.* The apparent lack of effect of *d*-amphetamine on the synthesis of DA in the nucleus accumbens and olfactory tubercle is perplexing, since the locomotor stimulating actions of this drug appear to be mediated via mesolimbic DA neurons [10]. To demonstrate that *d*-amphetamine exerts an action on DA neurons in these regions, the ability of this drug to prevent the decreases in DA synthesis induced by a monoamine oxidase inhibitor, nialamide, and a DA agonist, apomorphine, was examined. Nialamide decreased the rate of DOPA accumulation in the striatum, nucleus accumbens and olfactory tubercle, and amphetamine antagonized the nialamide-induced decrease in DA synthesis in all three regions (Fig. 2). Apomorphine also decreased the rate of DOPA accumulation in all regions, and again amphetamine effectively reversed this apomorphine-induced effect (Fig. 2). These results demonstrate that, although *d*-amphetamine does not alter basal rates of DA synthesis in nucleus accum-

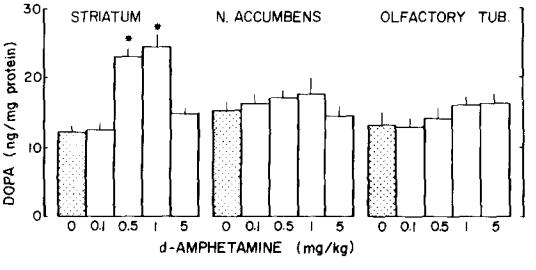

Fig. 1. Effect of *d*-amphetamine on the rate of DOPA accumulation in terminals of nigrostriatal and mesolimbic dopamine neurons. *d*-Amphetamine (0.1 to 5.0 mg/kg, s.c.) or saline vehicle (zero dose; hatched column) was administered 45 min and NSD 1015 (100 mg/kg, i.p.) was administered 30 min prior to killing the animal. Each column represents the mean, and the vertical line one S.E., of eight determinations. Key: (*) values that are significantly different ($P < 0.05$) from vehicle controls (zero dose).

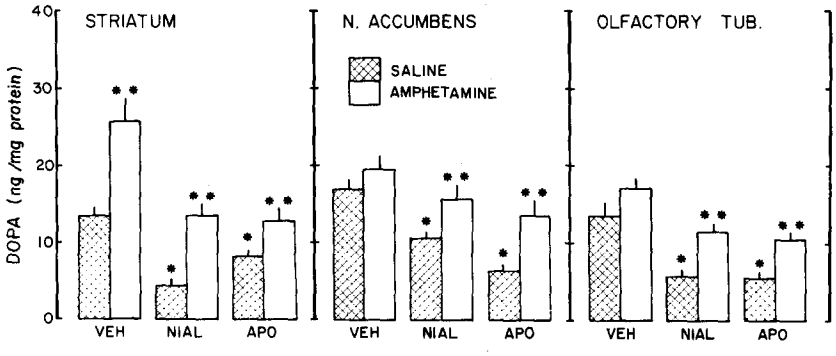

Fig. 2. Effect of *d*-amphetamine on the rate of DOPA accumulation in selected brain regions of rats pretreated with nialamide or apomorphine. *d*-Amphetamine (1 mg/kg) or saline was injected s.c. 45 min prior to sacrifice, and then all animals received NSD 1015 (100 mg/kg, i.p.) 30 min prior to sacrifice. In addition, these animals were pretreated with either nialamide (NIAL; 100 mg/kg, i.p.) 150 min prior to sacrifice, apomorphine (APO; 1 mg/kg, s.c.) 40 min prior to sacrifice, or with appropriate vehicles (VEH). Open columns represent the mean values from animals injected with *d*-amphetamine and hatched columns represent animals injected with a saline vehicle. Vertical lines represent one S.E. Key: (*) values significantly different ($P < 0.05$) from vehicle-saline controls; and (**), values in amphetamine-treated animals that are different from respective saline-treated controls.

bens and olfactory tubercle, it nevertheless is capable of exerting an action in these regions.

Effect of GBL on in vivo *DA synthesis in terminals of nigrostriatal and mesolimbic neurons.* GBL reduces the rate of firing of nigrostriatal and mesolimbic DA neurons as evidenced by the reduction of unit activity measured in the midbrain [8, 31]. As a result of this inhibition, GBL reduces the release of DA from these neurons [32] and this, in turn, is believed to reduce the stimulation of putative autoreceptors on DA nerve terminals that tonically inhibit DA synthesis. The end result is an acceleration of DA synthesis [2, 6]. Time and dose–response relationships for this effect of GBL in terminals of nigrostriatal and mesolimbic DA neurons are presented in Figs. 3 and 4. The results reveal that these neuronal systems are not equally responsive to the actions of GBL. An anesthetic dose of GBL (750 mg/kg) increased the rate of DOPA accumulation in the striatum, nucleus accumbens and olfactory tubercle, but this effect was more pronounced and longer lasting in the striatum (Fig. 3). Furthermore, the rate of DA synthesis in the striatum was increased at lower doses of GBL (300 mg/kg) than it was in the nucleus accumbens and olfactory tubercle (750 mg/kg; Fig. 4). These results suggest that either the nigrostriatal and mesolimbic neurons are differentially sensitive to the impulse-inhibiting actions of GBL or the mechanisms by which DA synthesis is regulated are not identical in these two neuronal systems.

Effect of d-*amphetamine on the GBL-induced increase in DA synthesis. d*-Amphetamine increases the release and blocks the reuptake of DA at nerve terminals (for review see Ref. 33). Corrodi and coworkers [34] reasoned that such an action would increase the concentration of DA in the synaptic cleft and would thereby activate neuronal feedback pathways which modulate DA neuronal firing. In agreement with this hypothesis, Bunney and coworkers [20] found that *d*-amphetamine markedly

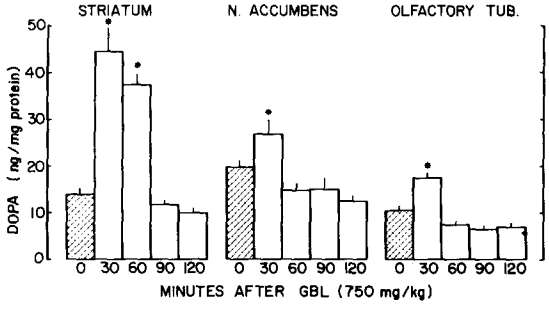

Fig. 3. Time course of the effect of GBL on DOPA accumulation in selected brain regions. GBL (750 mg/kg) or saline vehicle (zero time controls) was administered i.p. at 30, 60, 90 or 120 min prior to sacrifice, and NSD 1015 (100 mg/kg, i.p.) was administered 30 min prior to sacrifice. Columns represent means, and vertical lines one S.E., of eight determinations. Hatched columns represent values from saline-treated rats. Key: (*) values for GBL-treated animals that are significantly greater ($P < 0.05$) than saline (zero time) controls.

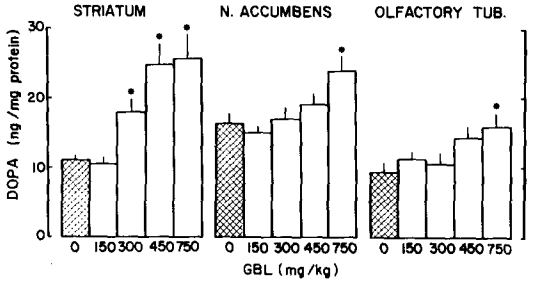

Fig. 4. Effect of various doses of GBL on DOPA accumulation in selected brain regions. GBL (150–750 mg/kg) or saline vehicle (zero dose) was injected i.p. 35 min prior to sacrifice and NSD 1015 (100 mg/kg, i.p.) was injected 30 min prior to sacrifice. Columns represent means, and vertical lines one S.E., of six to eight determinations. Key: (*) values that are significantly different ($P < 0.05$) from vehicle controls.

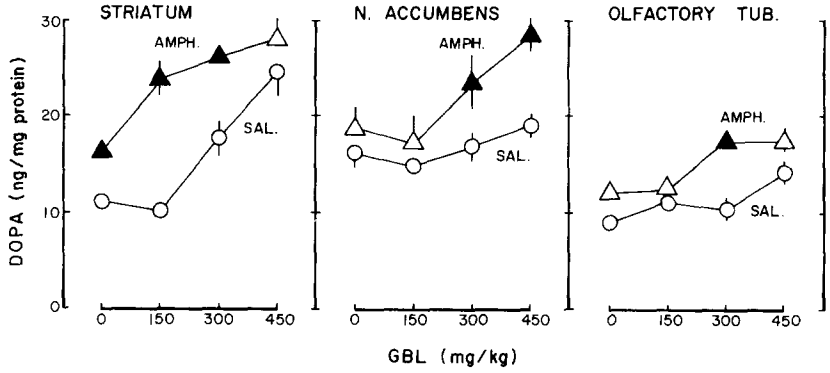

Fig. 5. Amphetamine enhancement of the GBL-induced increase in DOPA accumulation. Forty-five minutes prior to sacrifice, rats were injected s.c. with either *d*-amphetamine (0.125 mg/kg; △) or with saline (○). Ten minutes later (35 min prior to sacrifice) these animals were injected i.p. with GBL (150–450 mg/kg) or with saline (zero dose of GBL). Five minutes later (30 min prior to sacrifice) all animals were injected with NSD 1015 (100 mg/kg, i.p.). Each symbol represents the mean, and the vertical lines ± one S.E., as determined from eight animals. Solid symbols represent values from amphetamine-treated animals that are significantly different ($P < 0.05$) from the corresponding saline-pretreatment controls.

depressed the firing rate of nigrostriatal neurons in the zona compacta of the substantia nigra. Thus, a possible explanation for the observed regional difference in the action of amphetamine on DA synthesis may be that the neuronal feedback mechanisms which regulate firing in the nigrostriatal system are different or absent in mesolimbic regions [21]. To indirectly test this, the effect of amphetamine on the GBL-induced increase of DOPA accumulation was examined. Since both GBL and *d*-amphetamine decrease impulse traffic in ascending DA neurons, their effects on synthesis might be expected to be additive. Accordingly, the effect of a low dose of *d*-amphetamine (0.125 mg/kg) was examined on the GBL-induced increase of DOPA accumulation (Fig. 5). In the absence of GBL, this dose of amphetamine significantly increased DOPA accumulation in the striatum; it also markedly enhanced the effects of 150 and 300 mg/kg of GBL, effectively shifting the GBL dose–response curve to the left. *d*-Amphetamine *per se* was without effect on DOPA accumulation in the nucleus accumbens and olfactory tubercle but, when combined with a dose of GBL (300 mg/kg) that had no effect on its own, the rate of DOPA accumulation was increased significantly in both limbic brain regions. *d*-Amphetamine also increased the rate of DA synthesis in the nucleus accumbens after the administration of 450 mg/kg GBL. These results reveal that *d*-amphetamine enhances the GBL-induced acceleration of DA synthesis and that the striatum is more responsive and sensitive to this effect.

The enhancement of the GBL-induced increase in DA synthesis in the striatum by *d*-amphetamine noted in the present experiments is in contrast to the results of a previous study in which amphetamine reversed the GBL effect [35]. The different results were probably due to the doses of drugs employed. In the present study, a low dose of *d*-amphetamine (0.125 mg/kg, or 1/40th that used in the earlier study) and a submaximal dose of GBL were used to demonstrate an enhancement of the action of GBL. In the earlier study, a larger dose of *d*-amphetamine (5 mg/kg) was combined with a maximally effective dose of GBL (750 mg/kg). The inhibition of DA synthesis by the large dose of amphetamine may have resulted from a massive efflux of DA from nigrostriatal nerve terminals and the subsequent activation of the autoreceptors. Lower doses of *d*-amphetamine are apparently unable to release sufficient DA in the GBL-treated animal to activate these receptors. Even a moderate dose of *d*-amphetamine (1 mg/kg, s.c., 45 min) did not alter the increased rate of DOPA accumulation in the striatum which was induced by a maximally effective dose of GBL (750 mg/kg). Values for DOPA accumulation (ng per mg protein per 30 min) were: control, 9.4 ± 0.6; GBL, 20.4 ± 1.2; *d*-amphetamine, 24.6 ± 1.7; and GBL + *d*-amphetamine, 24.7 ± 1.1 (mean ± 1 S.E., N = 8).

Apomorphine reversal of the GBL-induced increase in DA synthesis. One possible explanation for the differential action of *d*-amphetamine and GBL in the nigrostriatal and mesolimbic DA systems is that autoreceptors on the terminals of these neurons have different sensitivities. If auroreceptors on mesolimbic DA neurons are more sensitive than those on nigrostriatal neurons, one might expect that released DA would have a relatively greater inhibitory role in regulating synthesis in the former neurons. Studies were undertaken to examine the sensitivities of DA autoreceptors on mesolimbic and nigrostriatal nerve terminals. Since GBL (750 mg/kg, i.p.) inhibits DA neuronal impulse flow, the influence of presynaptic autoreceptor activation on synthesis can be examined directly [22]. The ability of apomorphine to antagonize the GBL-induced increase of DOPA accumulation is depicted in Fig. 6. In the striatum, the GBL-induced increase in DOPA accumulation was decreased significantly after doses of 0.1 mg/kg or greater of apomorphine, while in the nucleus accumbens and olfactory tubercle the GBL-induced increase was decreased significantly at doses of apomorphine as low as

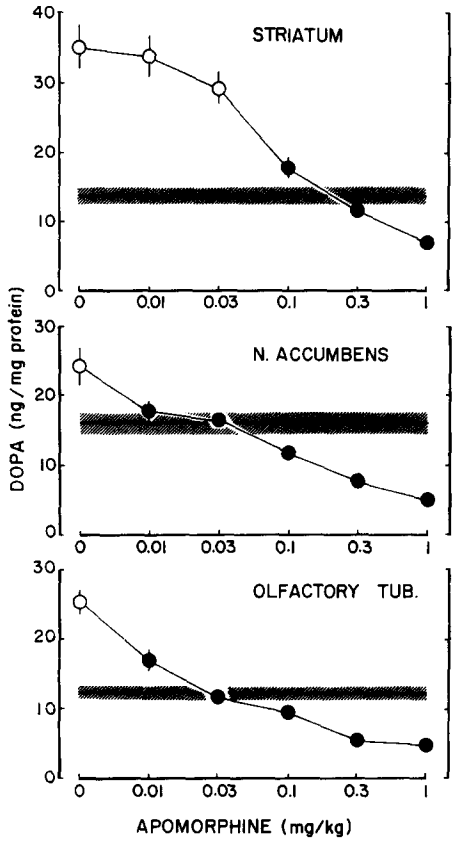

Fig. 6. Reversal of the GBL-induced increase in DOPA accumulation by apomorphine. Apomorphine (0.01 to 1.0 mg/kg, s.c.) and GBL (750 mg/kg, i.p.) were administered 45 and 35 min prior to sacrifice; NSD 1015 (100 mg/kg, i.p.) was administered 30 min prior to sacrifice. Symbols represent the means, and vertical lines ± one S.E., of eight determinations; solid symbols represent those values that are significantly different ($P < 0.05$) from GBL alone (zero dose apomorphine). The horizontal lines and shaded areas represent means ± one S.E. in control animals that received NSD 1015 alone. Where no vertical lines are depicted the S.E. is less than the radius of the symbol.

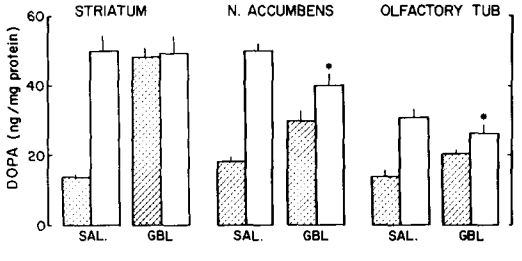

Fig. 7. Effect of haloperidol on the GBL-induced increase in DOPA accumulation. Haloperidol (0.1 mg/kg) or its 0.3% tartaric acid vehicle was injected s.c. 60 min prior to sacrifice, and GBL (750 mg/kg) or its saline vehicle was injected i.p. 35 min prior to sacrifice; NSD 1015 (100 mg/kg, i.p.) was injected into all animals 30 min prior to sacrifice. Columns represent means, and vertical lines one S.E., of eight determinations. Key: hatched columns, tartaric acid vehicle; open columns, haloperidol; and (*) values in haloperidol–GBL-treated animals that are significantly different ($P < 0.05$) from vehicle–GBL-treated animals.

0.01 mg/kg. These results indicate that DA autoreceptors on terminals of mesolimbic neurons are more sensitive to the actions of a DA agonist, and probably to released DA.

Effect of haloperidol on the GBL-induced increase in DA synthesis. The apparently greater sensitivity of presynaptic DA receptors on mesolimbic neurons may have been responsible for the reduced magnitude of the GBL-induced increase in DOPA accumulation in the nucleus accumbens and olfactory tubercle. Even though the release of DA is probably decreased to the same extent by GBL in limbic regions and striatum, the low concentrations of DA at the presynaptic receptors on nerve terminals in the former regions are still sufficient to maintain synthesis at basal levels. Since haloperidol blocks both pre- and postsynaptic DA receptors, the rate of DOPA accumulation in limbic regions should be increased to a greater extent after the concurrent administration of haloperidol and GBL than after GBL alone. The results illustrated in Fig. 7 confirm this proposal. The administration of haloperidol prior to GBL resulted in greater stimulation of DOPA accumulation compared to GBL alone in the nucleus accumbens and olfactory tubercle but not in the striatum. The results suggest that the lack of effectiveness of GBL in the nucleus accumbens and olfactory tubercle resulted from the greater sensitivity of DA presynaptic receptors on the terminals of mesolimbic neurons in these regions so that the reduced amount of DA released after the administration of GBL was still able to activate autoreceptors on terminals of mesolimbic neurons, but not on the less sensitive presynaptic receptors on nigrostriatal DA neurons.

DISCUSSION

Dynamic events believed to occur at a "typical" DA neuronal terminal are depicted schematically in Fig. 8. Within the terminal, DA is synthesized in a two-step process. In the first and rate-limiting step, tyrosine is converted to DOPA, a reaction that is catalyzed by tyrosine hydroxylase. In the second step, the newly synthesized DOPA is rapidly decarboxylated by aromatic L-amino acid decarboxylase to form DA, which can then be stored or released

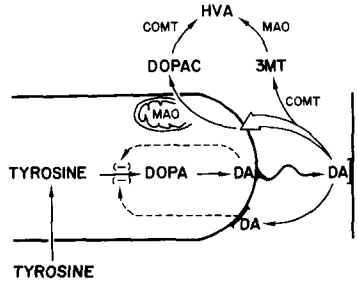

Fig. 8. Schematic diagram of neurochemical events occurring at a dopaminergic neuronal terminal. Abbreviations: HVA, homovanillic acid; 3MT, 3-methoxytyramine; COMT, catechol-*O*-methyltransferase; MAO, monoamine oxidase; DOPAC, dihydroxyphenylacetic acid; DOPA, dihydroxyphenylalanine; and DA, dopamine.

into the synaptic cleft in response to the arrival of nerve action potentials or the actions of drugs. In the synaptic cleft, DA is free to interact with postsynaptic or putative presynaptic receptors. The DA is then actively transported back into the nerve terminals where it can enter a "releasable pool", but most of the recaptured amine appears to be oxidatively deaminated to dihydroxyphenylacetic acid (DOPAC) by intraneuronal monoamine oxidase.

The synthesis of DA appears to be regulated by at least two mechanisms (for review see Ref. 2). In the first of these (end-product feedback inhibition), a "strategic" intraneuronal pool of DA inhibits tyrosine hydroxylase, presumably by antagonizing the binding of a pteridine cofactor to the enzyme. The rate of DA synthesis is inversely proportional to the size of this pool. As DA is released from the neuron in response to impulse traffic, the intracellular pool of this amine is presumably reduced, resulting in the removal of the end-product inhibition of tyrosine hydroxylase. Consequently, the rate of DA synthesis increases. In this manner synthesis keeps pace with release so that steady-state concentrations of DA do not decline when impulse traffic in the neurons increases. Furthermore, the rate of synthesis of DA in nerve terminals can be used to estimate the activity of these neurons [6].

Tyrosine hydroxylase is also believed to be controlled by DA receptors located on the nerve terminal (presynaptic autoreceptors); this has been referred to as "short loop feedback" control. The exact mechanism involved is not well understood, but it appears that activation of these receptors inhibits DA synthesis. On the other hand, during periods when these receptors are not activated, the rate of DA synthesis increases. This latter effect occurs after the administration of GBL, a drug that depresses impulse traffic in the ascending DA neurons. Under the influence of this drug, DA is not released and presynaptic receptors are not activated. As a result, the presynaptic receptor-mediated brake on tyrosine hydroxylase is removed and DA synthesis increases. The GBL-induced increase of DA synthesis can be reversed by administering a drug, such as apomorphine, which mimics the action of DA at the autoreceptors. This action of apomorphine, in turn, can be blocked by drugs, such as haloperidol, which block DA autoreceptors. Since GBL blocks the influence of the postsynaptic DA receptor-mediated changes via the long neuronal feedback loops, the action of apomorphine and haloperidol in GBL-pretreated animals must be restricted to the DA receptor-mediated mechanisms located at the nerve terminals.

How does *d*-amphetamine fit into the scheme of events occurring at the DA neuronal terminal? This drug is believed to exert its central stimulant action by facilitating the release of DA, and by inhibiting the neuronal reuptake of the released amine (see review in Ref. 33). As a result, one might expect the "strategic" intraneuronal pool of DA which regulates synthesis to be reduced, thereby diminishing end-product inhibition of tyrosine hydroxylase. Consequently, DA synthesis would increase. On the other hand, *d*-amphetamine might be expected to decrease DA synthesis by increasing the concentration of DA in the synaptic cleft and thereby activating pre- and postsynaptic receptors. As a result of postsynaptic receptor stimulation, the inhibitory neuronal feedback loops would be activated causing reduced impulse traffic in the ascending DA neurons and an associated decrease in DA synthesis. In addition, an increase of presynaptic DA receptor occupation would activate these receptor-mediated mechanisms to inhibit DA synthesis. Obviously the effects of *d*-amphetamine on DA synthesis are complex, with the end result being dependent upon the mean response to several different actions of the drug. Bunney *et al.* [20] have suggested that low doses of amphetamine reduce impulse flow in nigrostriatal DA neurons via the neuronal feedback loops whereas the effect after higher doses reflects activation of DA receptors on nigral cell bodies or dendrites.

A question to be considered is why does *d*-amphetamine increase DA synthesis in nigrostriatal nerve terminals in the striatum, but not in mesolimbic nerve terminals in the olfactory tubercle and nucleus accumbens ([12–14]: Fig. 1)? *d*-Amphetamine can apparently increase DA synthesis in the terminals of both nigrostriatal and mesolimbic DA neurons, but in the latter this could be demonstrated only when synthesis was depressed by pretreating with the monoamine oxidase inhibitor nialamide (increasing end-product feedback inhibition) or with a DA agonist (activating pre- or postsynaptic DA receptors) (Fig. 2). Obviously, DA synthesis in both neuronal systems responds to end-product inhibition and to DA receptor-mediated control mechanisms (see also Ref. 7), but there must be some qualitative difference in the responses of mesolimbic and nigrostriatal DA neurons which becomes manifest when *d*-amphetamine is administered. (Similar differences are also seen after the administration of morphine, which also causes a more pronounced increase of DA synthesis in the striatum than in limbic forebrain regions [36].) The finding that *d*-amphetamine reverses the apomorphine-induced depression of DA synthesis appears to be unexpected in that both drugs should activate presynaptic DA receptors, apomorphine by a direct action and *d*-amphetamine by facilitating DA release. One possible explanation for the paradoxical result could be that *d*-amphetamine releases DA from an intraneuronal pool which, in turn, removes end-product inhibition of DA synthesis and thereby antagonizes the presynaptic receptor-mediated decrease of DA synthesis induced by apomorphine.

Wang [21] has clearly demonstrated differences in the mechanism by which *d*-amphetamine alters impulse traffic in mesolimbic and nigrostriatal DA neurons, and this difference may play a role in the *d*-amphetamine-induced changes in DA synthesis. Nevertheless, differential regulation of DA synthesis in terminal regions of mesolimbic and nigrostriatal DA neurons is also observed when impulse traffic in these neurons is interrupted, suggesting differences in autoreceptor mechanisms. That is, after the administration of GBL the resulting acceleration of DA synthesis shows regional specificity with an effect in the striatum occurring at lower doses and exhibiting a greater increase over a longer duration than

that seen in nucleus accumbens or olfactory tubercle (Figs. 3 and 4). Roth and Nowycky [8] have shown that doses of apomorphine required to reverse the GBL-induced increase of DA synthesis are lower in the olfactory tubercle than in the striatum. The data reported in Fig. 6 confirm the fact that autoreceptors on terminals of mesolimbic neurons are more sensitive to a DA agonist than are those on nigrostriatal neurons. It is interesting to note, however, that the difference in the sensitivity of DA autoreceptors on nigrostriatal and mesolimbic neurons is small when compared to the differences between the characteristics of autoreceptors on these neurons and those on mesocortical DA neurons. These latter neurons appear to have very insensitive autoreceptors regulating DA synthesis [37].

The apparently greater sensitivity of DA autoreceptors on mesolimbic DA neurons may be responsible for the decreased abilities of amphetamine and GBL to alter DA synthesis in regions containing the terminals of these neurons. Although both drugs reduce impulse traffic in nigrostriatal and mesolimbic DA neurons, a dramatic increase in synthesis of DA is observed only in the striatum. Since DA autoreceptors appear to be more sensitive to released DA in mesolimbic DA neurons, a comparable reduction in firing and release of DA does not elicit the same increase in synthesis in limbic versus striatal forebrain regions. That is, the more sensitive autoreceptors on mesolimbic DA neurons may still respond to the reduced amount of DA released, whereas the less sensitive autoreceptors on nigrostriatal neurons are not activated so that the short loop feedback mechanism regulating synthesis in these latter neurons is disrupted. The greater sensitivity of autoreceptors on mesolimbic DA neurons, therefore, may be the reason that DA synthesis in limbic forebrain regions is more tightly regulated. Drug-induced changes in DOPA accumulation in nucleus accumbens and olfactory tubercle are generally smaller in magnitude and shorter in duration than are those in the striatum [7, 14, 36]. Differences in the mechanisms by which DA synthesis is regulated in terminals of nigrostriatal and mesolimbic DA neurons, although subtle, may explain why amphetamine (and other drugs such as morphine) exert a dose-dependent differential biochemical action on these two neuronal systems.

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