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# DOPAMINERGIC NEURONS—ALTERATION IN THE SENSITIVITY OF TYROSINE HYDROXYLASE TO INHIBITION BY ENDOGENOUS DOPAMINE AFTER CESSATION OF IMPULSE FLOW*

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**Abstract**—Inhibition of firing in the nigro-neostriatal dopamine system causes a rapid increase in the steady state levels of dopamine and an apparent activation *in vivo* of tyrosine hydroxylase. Although the apparent activity of tyrosine hydroxylase returns to normal after dopamine levels have been increased, the elevated dopamine levels do not appear to inhibit tyrosine hydroxylase below control levels. In contrast, when dopamine levels are increased by administration of a monoamine oxidase inhibitor, there is a significant reduction in striatal tyrosine hydroxylase activity as measured by the accumulation of dihydroxyphenylalanine (DOPA) after administration of a DOPA decarboxylase inhibitor. These results suggest that a blockade of impulse flow in the dopaminergic neurons causes a decrease in the sensitivity of striatal tyrosine hydroxylase to inhibition by elevated levels of dopamine. Administration of dopamine agonists reverses this effect and restores to normal the sensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine. This effect of the dopamine agonists is prevented or reversed by administration of drugs which block dopamine receptors. Thus, the dopamine agonists appear to alter dopamine synthesis in the absence of impulse flow by interacting at a site, possibly on the presynaptic side of the dopamine terminal, which is similar to the post-synaptic dopaminergic receptor and which appears capable of altering the sensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine.

Many laboratories have described the rapid and marked increase in neostriatal dopamine levels which occurs after impulse flow has been inhibited in the nigro-neostriatal pathway [1–8]. This increase can be elicited by cerebral hemisection [6], by lesion of the substantia nigra or median forebrain bundle [1, 2, 4, 5, 8, 9], by intraventricular injection of 6-OH dopamine [10] and by administration of gamma-butyrolactone (GBL) [11–14], a drug known to inhibit the firing of the nigro-neostriatal neurons [7].

The increase in neostriatal dopamine levels following inhibition of impulse flow in the nigro-neostriatal pathway is accompanied by an apparent activation of tyrosine hydroxylase as measured by the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after administration of a DOPA decarboxylase inhibitor [6, 15, 16] and by increased conversion of [$^3$H]tyrosine to DOPA and dopamine [8, 17]. The hypothesis that tyrosine hydroxylase activity is normally regulated by end-product feedback inhibition is inconsistent with this observation that dopamine synthesis can be elevated while endogenous levels of dopamine are also increasing.

To investigate this phenomenon further we have studied the ability of drugs which interact with dopamine receptors to modify the change in dopamine synthesis following inhibition of impulse flow. These effects have been compared with the changes in dopamine synthesis occurring after elevation of dopamine by inhibition of monoamine oxidase (MAO). The results suggest that the increase in dopamine synthesis following blockade of impulse flow may be due to a decrease in the inhibitory effects of intraneuronal dopamine on tyrosine hydroxylase activity. Moreover, dopamine agonists appear to reverse this effect by interacting with dopamine receptors, most probably located on the presynaptic side of the synapse [15, 18].

## MATERIALS AND METHODS

Male Sprague–Dawley rats (200–350 g) obtained from Charles River Inc. were used throughout the experiments. Drugs were administered interperitoneally (i.p.). Gamma-butyrolactone (GBL) (Matheson, Coleman & Bell) was used in preference to the sodium salt of gamma-hydroxybutyric acid (GHB), since it is more rapidly and uniformly absorbed following i.p. injection. Other drugs used were RO4-4602 (seryl-trihydroxybenzylhydrazine; Hoffmann–LaRoche, Inc.), *d*-amphetamine sulfate (K & K Laboratories, Inc.), apomorphine HCl, phenoxybenzamine and chlorpromazine (Smith, Kline & French Laboratories), haloperidol (McNeil Laboratories), pargyline HCl (Abbott Laboratories), promethazine

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(Wyeth Laboratories), propranolol (Ayerst Laboratories) and 1-(2-pyrimidyl)-piperonyl-piperazine (ET-495; Laboratories Servier).

The method of Carlsson *et al.* [6] was used to investigate the effects of various drug treatments on tyrosine hydroxylase activity *in vivo*. We have previously demonstrated that the accumulation of DOPA in the striatum is linear for at least 60 min after the inhibition of DOPA decarboxylase with RO4-4602 (800 mg/kg) [16]. In the following experiments the accumulation of DOPA after various pretreatments has been used as an approximation of the relative effects of these treatments on the activity *in vivo* of tyrosine hydroxylase. After various drug pretreatments, the animals were given 800 mg/kg of RO4-4602, a rapidly acting inhibitor of DOPA decarboxylase, and killed by decapitation 30 min later. The striata were rapidly dissected over ice as described by Bunney *et al.* [19] and kept frozen at $-70°$ for up to 3 days before analysis. DOPA was isolated and measured fluorometrically, essentially by the technique of Kehr *et al.* [20] with some modifications as described by Walters and Roth [16].

Dopamine determinations were performed on rats which did not received RO4-4602. A modification of the method of Lavery and Taylor [21] was used as described in Walters and Roth [13]. Data were analyzed using a two-tailed *t*-test with a criterion for significance of $P < 0.05$.

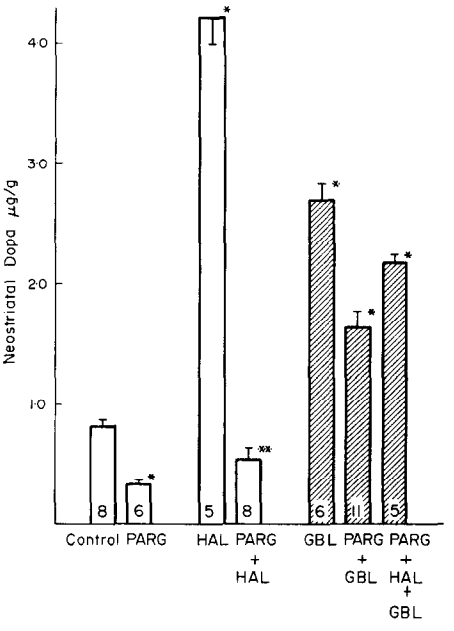


Fig. 1. Effect of pargyline on the accumulation of neostriatal DOPA after inhibition of firing. All animals were treated with 800 mg/kg of RO4-4602 30 min before sacrifice. GBL (750 mg/kg) was administered i.p. 35 min before sacrifice, haloperidol (HAL, 1 mg/kg) was administered 45 min before sacrifice and pargyline (PARG, 75 mg/kg) was administered 3 hr before sacrifice, where indicated. The bars represent the S. E. M. and the numbers in the columns indicate the number of individual determinations. A single asterisk indicates significantly different from control, $P < 0.001$. A double asterisk indicates significantly different from control, $P < 0.02$.

## RESULTS

In confirmation of previous findings [15,16], inhibition of impulse flow produced by the administration of 750 mg/kg of GBL 5 min before treatment with RO4-4602 caused a marked increase in the 30-min accumulation of neostriatal DOPA, as compared with control (Fig. 1). Over this time-period there is a significant increase in the levels of dopamine in the neostriatum as well [13]. Since the increased dopamine levels caused by inhibition of impulse flow do not appear to have an inhibitory effect on the apparent activity of tyrosine hydroxylase, it was of interest to determine whether tyrosine hydroxylase activity would be altered by a pargyline-induced increase in dopamine.

When RO4-4602 was administered 2·5 hr after administration of pargyline and the rats were sacrificed 30 min later, the accumulation of DOPA was found to be markedly decreased to approximately 40 per cent of control (Fig. 1). At this time, 3 hr after pargyline administration, the levels of dopamine in the neostriatum were significantly increased (Fig. 2). The decreased accumulation of DOPA observed after dopamine levels were increased by MAO inhibition was not due to a direct effect of pargyline on dopamine synthesis, as the accumulation of DOPA during the first 30 min after administration of this drug was not significantly different from control ($0.66 \pm 0.05$ μg/g, mean ± S. E. M.; $N = 5$). In contrast to the effects observed after inhibition of impulse flow, this apparent decrease in the activity of tyrosine hydroxylase, after dopamine levels have been increased by MAO inhibition, does seem consistent with the hypothesis that endogenous dopamine exerts a negative feedback effect on tyrosine hydroxylase.

The apparent inhibition of tyrosine hydroxylase activity caused by the increase in dopamine after pargyline administration is even more evident with haloperidol treatment (Fig. 1). Administration of 1 mg/kg of haloperidol alone caused a marked increase in the 30-min accumulation of DOPA. This was expected, as haloperidol has been shown to cause a marked increase in the firing of the nigro-neostriatal dopamine neurons [19] and a concomitant increase in dopamine turnover [22]. However, when endogenous levels of dopamine were elevated by administration of pargyline 2·5 hr before haloperidol administration, the 30-min accumulation of DOPA was only 12 per cent of that observed after haloperidol alone.

Pargyline pretreatment was much less effective in inhibiting tyrosine hydroxylase in the absence of impulse flow. When GBL was administered 5 min before the DOPA decarboxylase inhibitor, after dopamine levels had been increased by pargyline pretreatment, DOPA accumulation was less inhibited than in the animals receiving only pargyline or pargyline plus haloperidol (Fig. 1). DOPA accumulation was 60 per cent of that observed in the animals receiving GBL alone. When GBL was administered to animals treated with pargyline and haloperidol, the accumulation of DOPA was again less inhibited than in those animals receiving pargyline and haloperidol alone.

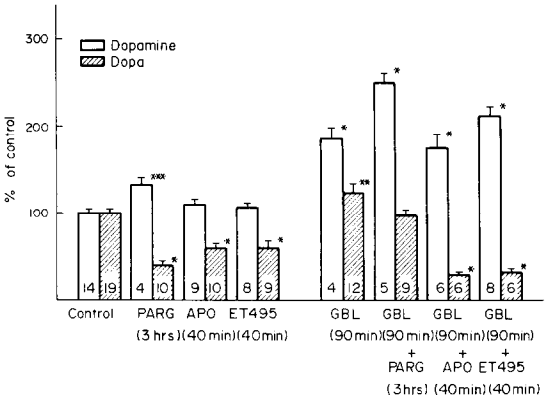


Fig. 2. Effect of MAO inhibition and dopamine agonists on dopamine and DOPA levels after inhibition of impulse flow. For all DOPA determinations, RO4-4602 was administered i.p. 30 min before sacrifice. Dopamine (DA) determinations were performed on rats which did not receive RO4-4602. GBL (800 mg/kg) was administered i.p. 90 min before sacrifice; apomorphine (APO, 2 mg/kg) and ET-495 (10 mg/kg) were administered 40 min before sacrifice, where indicated. The bars represent the S. E. M. and the numbers in the columns indicate the number of individual determinations. Percentages were determined on the basis of individual controls. Pooled dopamine controls were 9·42 ± 0·37 μg/g (N = 8), and DOPA controls were 0·80 ± 0·03 μg/g (N = 19; mean ± S.E.M.). A single asterisk indicates significantly different from control, $P < 0·001$. A double asterisk indicates significantly different from control, $P < 0·05$. A triple asterisk indicates significantly different from control, $P < 0·002$.

One possible explanation for these results was that inhibition of firing reduced the ability of endogenous dopamine to exert an inhibitory effect on the activity of tyrosine hydroxylase. To investigate this further, the accumulation of DOPA was measured during the period when dopamine levels were maximally elevated by GBL treatment (Fig. 2). RO4-4602 was administered 60 min after GBL treatment and the animals were sacrificed 30 min later. It has been previously reported [16] that dopamine levels are maximally increased after inhibition of impulse flow at this time. Dopamine synthesis rates appear to return to normal but are not significantly different from control despite the existing 80–100 per cent increase in dopamine (Fig. 2). This finding is in contrast to the observation that the smaller increase in dopamine produced by pargyline pretreatment has a marked inhibitory effect on DOPA accumulation. In another group of animals, the pargyline and 90-min GBL treatments were combined, and levels of dopamine were considerably higher than those after pargyline alone, but again, the accumulation of DOPA was not significantly different from control (Fig. 2). As with the short-term GBL treatment, increased dopamine levels in the absence of impulse flow did not exert an inhibitory effect on the apparent activity of tyrosine hydroxylase.

It has been shown that dopamine agonists can block the increase in dopamine synthesis which occurs after inhibition of impulse flow [16] and it has been suggested that these agonists may exert this effect by acting at a site similar to the post-synaptic dopamine receptor but located on the presynaptic side of the synapse [18]. If inhibiting impulse flow in the dopaminergic neurons does alter the inhibitory effect of endogenous dopamine on tyrosine hydroxylase activity, it seemed possible that presynaptic receptor stimulation might prevent this alteration and might also reverse it once it had occurred. To investigate this, apomorphine was administered after dopamine levels had been elevated by pretreatment with GBL (Fig. 2). The subsequent 30-min accumulation of DOPA was significantly less than control, less than that observed after apomorphine alone, and also significantly less than that observed when apomorphine and GBL were administered shortly before the DOPA decarboxylase inhibitor (Fig. 3) when dopamine levels were not yet maximally increased [13]. Similar results were obtained when ET-495, another dopamine agonist, was administered in place of apomorphine (Fig. 2). Since the apparent activity of tyrosine hydroxylase was reduced more when DA levels were higher, it appears that the administration of a dopamine agonist after inhibition of impulse flow results in an apparent restoration in the ability of endogenous dopamine to inhibit tyrosine hydroxylase effectively.

As shown in Table 1, the effect of apomorphine on the synthesis of dopamine after GBL administration was blocked by haloperidol, a dopamine receptor blocking drug. When haloperidol was

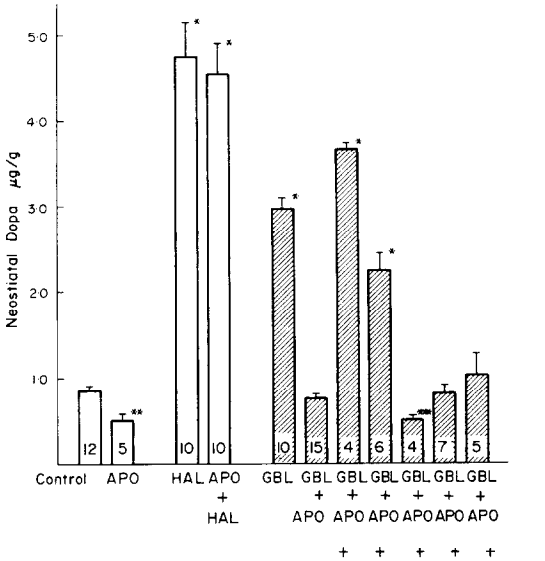


Fig. 3. Effect of apomorphine and other drugs on neostriatal DOPA accumulation following inhibition of impulse flow. All animals received 800 mg/kg of RO4-4602, i.p. 30 min before sacrifice. GBL (750 mg/kg) was administered i.p. 35 min before sacrifice, apomorphine (APO, 2 mg/kg) was administered i.p. 40 min before sacrifice and haloperidol (HAL, 1 mg/kg), chlorpromazine (CPZ, 10 mg/kg), promethazine (PROM, 10 mg/kg), propranolol (PROPAN, 5 mg/kg) and phenoxybenzamine (PBA, 25 mg/kg) were administered 45 min before sacrifice, where indicated. The bars represent the S. E. M. and the numbers in the columns indicate the number of individual determinations. A single asterisk indicates significantly different from control, $P < 0·001$. A double asterisk indicates significantly different from control, $P < 0·01$. A triple asterisk indicates significantly different from control, $P < 0·005$.

Table 1. Effect of haloperidol on striatal DOPA accumulation after apomorphine and 90-min GBL treatment*

| Treatment | DOPA (μg/g) | N |
|---|---|---|
| Control | 0·96 ± 0·13 | 6 |
| GBL, 90 min | 0·92 ± 0·06 | 5 |
| HAL, 45 min | 5·35 ± 0·16† | 4 |
| GBL, 90 min + HAL, 45 min | 1·20 ± 0·06 | 8 |
| GBL, 90 min + APO, 40 min | 0·24 ± 0·03† | 10 |
| GBL, 90 min + HAL, 45 min + APO (40 min) | 0·99 ± 0·04 | 4 |

* All animals received 800 mg/kg of RO4-4602 30 min before sacrifice. GBL (750 mg/kg), haloperidol (HAL, 1 mg/kg) and apomorphine (APO, 2 mg/kg) were administered i.p. or as indicated.

† Significantly different from control, $P < 0·005$.

administered 5 min before apomorphine and 45 min after GBL treatment, the accumulation of DOPA was similar to that observed after GBL alone.

Figure 3 indicates that dopamine receptor blocking agents can also prevent the effect of apomorphine when both agonist and antagonist are administered before impulse flow is blocked. Apomorphine (2 mg/kg) alone, administered 10 min before treatment with RO4-4602, caused a signiificant decrease in DOPA accumulation as compared with control. The administration of apomorphine had little effect on the accumulation of DOPA observed after haloperidol treatment, but when apomorphine was administered to animals 5 min before impulse flow was inhibited by GBL treatment, the increased accumulation of DOPA normally observed after GBL administration was blocked.

The apomorphine-induced blockade of the apparent activation of tyrosine hydroxylase caused by inhibition of impulse flow was reversed by pretreatment with haloperidol (1 mg/kg) or chlorpromazine (10 mg/kg). As illustrated in Fig. 3, the effect of apomorphine on the accumulation of striatal DOPA after administration of GBL was not reversed by pretreatment with phenoxybenzamine (25 mg/kg) or propranolol (5 mg/kg), both peripheral adrenergic-blocking agents, or by promethazine (10 mg/kg), a phenothiazine which, unlike the antipsychotic phenothiazines, does not block post-synaptic dopamine receptors [19].

Figure 4 shows similar results obtained with ET-495, another dopamine receptor stimulating agent [23]. Like apomorphine, ET-495 (10 mg/kg), administered before GBL, blocked the increase in DOPA accumulation normally produced by GBL administration, and this block was reversed by pretreatment with 1 mg/kg of haloperidol.

A treatment thought to promote an increase in the release of dopamine into the synaptic cleft also prevented the increase in DOPA accumulation after inhibition of impulse flow. The administration of *d*-amphetamine (5 mg/kg) inhibited the increase in DOPA accumulation following GBL administration (Fig. 4). This effect was reversible as well; it was blocked by haloperidol pretreatment.

## DISCUSSION

During the first 0·5 hr after inhibition of impulse flow in the dopaminergic nigro-neostriatal neurons, the activity *in vivo* of striatal tyrosine hydroxylase appears to be markedly increased [6, 15, 16]. Over this period, there is an increased incorporation of [$^3$H]tyrosine into dopamine [8, 17] and an increased accumulation of DOPA after administration of a DOPA decarboxylase inhibitor [6, 15, 16]. Once dopamine levels have risen by approximately 80–100 per cent after impulse flow inhibition, they remain at this elevated level until impulse flow is restored or the terminals degenerate [16]. Similar effects are not observed after inhibition of impulse flow in CNS neurons containing norepinephrine [24] or serotonin [6, 25].

The hypothesis that the activity of tyrosine hydroxylase is normally decreased by end-product inhibition is inconsistent with the apparent increase in striatal tyrosine hydroxylase activity observed after inhibition of impulse flow. Moreover, once dopamine levels have increased to a new steady-state level, tyrosine hydroxylase activity appears to return to normal [16], but the increased transmitter levels do not inhibit the enzyme.

In the intact firing system, however, an increase in striatal dopamine levels does appear to inhibit the apparent activity of tyrosine hydroxylase *in vivo*, as the experiments with MAO inhibition indicate. Striatal MAO appears to be, in part, intraneuronal [26]

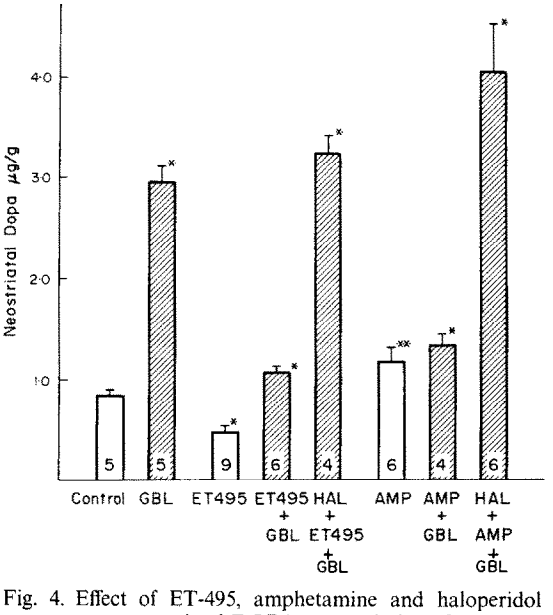


Fig. 4. Effect of ET-495, amphetamine and haloperidol treatment on neostriatal DOPA accumulation after inhibition of firing. All animals received 800 mg/kg of RO4-4602 30 min before sacrifice. GBL (750 mg/kg) was administered i.p. 35 min before sacrifice, ET-495 (10 mg/kg) and amphetamine (AMP, 5 mg/kg) were administered i.p. 40 min before sacrifice and haloperidol (HAL, 1 mg/kg) was administered i.p. 45 min before sacrifice, where indicated. The bars represent the S. E. M. and the numbers in the columns indicate the number of individual determinations. A single asterisk indicates significantly different from control, $P < 0·001$. A double asterisk indicates significantly different from control. $P < 0·005$.

and inhibition of MAO would presumably cause an increase in the levels of intraneuronal dopamine. The finding that the increase in dopamine caused by MAO inhibition is accompanied by a marked decrease in the apparent activity of tyrosine hydroxylase supports the idea that under these conditions tyrosine hydroxylase activity is decreased by end-product inhibition. When impulse flow was blocked by the administration of GBL after pargyline pretreatment, however, the increased dopamine levels appeared to exert much less of an inhibitory effect on striatal tyrosine hydroxylase activity.

One explanation for these findings is that the intraneuronal localization of dopamine is altered by inhibition of impulse flow in such a way that it is no longer able to exert an inhibitory effect on tyrosine hydroxylase. This would mean, however, relocating not only newly synthesized dopamine, but also, in the pargyline experiments, the dopamine which had accumulated intraneuronally during the period of MAO inhibition. Another explanation would be that a decrease in the sensitivity of tyrosine hydroxylase to the inhibitory effects of dopamine occurs subsequent to impulse flow inhibition [27]. Evidence *in vitro* for such a change in tyrosine hydroxylase following inhibition of impulse flow by both mechanical and pharmacological means is presented in the following paper [28].

If the increase in dopamine synthesis occurring after inhibition of impulse flow is due to an alteration in the ability of interneuronal dopamine to exert an inhibitory effect on tyrosine hydroxylase, it seems possible that the dopamine agonists may act by reversing this alteration. The administration of a dopamine agonist after inhibition of impulse flow resulted in an apparent restoration of the sensitivity of tyrosine hydroxylase to inhibition by endogenous dopamine. In the presence of apomorphine, the activity of tyrosine hydroxylase was decreased more when dopamine levels were highest. The agonists do not appear to be interacting directly with striatal tyrosine hydroxylase as they do not significantly inhibit the enzyme when added to the incubation medium in concentrations as high as $10^{-4}$ M [28]. Also, their effects on dopamine synthesis in the presence or absence of impulse flow are blocked by haloperidol. In contrast, the inhibitory effect of increased intraneuronal dopamine caused by MAO inhibition is not blocked by haloperidol. Haloperidol administration also does not cause a substantial increase in DOPA accumulation 60–90 min after GBL treatment (Table 1). This suggests that leakage and accumulation of dopamine in the synaptic cleft are not important factors in causing dopamine synthesis to return to control levels at this time after GBL treatment. Perhaps intraneuronal dopamine levels eventually become sufficiently high to exert some limited direct inhibitory effects on tyrosine hydroxylase, reversing the acceleration of dopamine synthesis observed during the first 30 min after inhibition of impulse flow.

It has been hypothesized that the ability of apomorphine to prevent the increase in DOPA accumulation after inhibition of impulse flow is related to the agonist's ability to stimulate dopamine receptors located either pre- or post-synaptically on the dopamine terminal [18]. This hypothesis is further supported by the observation that pretreatment with ET-495, another dopamine agonist, and amphetamine, a dopamine-releasing compound, also blocks the increase in DOPA accumulation after GBL and that these effects can be reversed by receptor blocking agents. Thus the dopamine agonists appear able to block and reverse the apparent activation of tyrosine hydroxylase which occurs when impulse flow is inhibited in the dopamine neurons by interacting at a presynaptic site analogous to the post-synaptic dopamine receptor. Recent studies *in vitro* in our laboratory [27,28] suggest that an alteration in calcium flux may play an important role in these changes in tyrosine hydroxylase activity.

The existence of a presynaptic receptor mechanism which modulates presynaptic function was first suggested to explain findings in the peripheral nervous system. It was observed by many investigators that drugs such as phenoxybenzamine and phentolamine, which blocked α-adrenergic receptors, also caused an increase in the amount of norepinephrine released from noradrenergic neurons in a variety of preparations [29–39]. Several mechanisms were proposed to account for this phenomenon [29, 32, 34, 40, 41]. Among these was the suggestion that the presynaptic portion of the noradrenergic terminal has receptors similar to α-receptors, which modulate the amount of NE released per nerve impulse [30, 39, 42, 43] by mediating changes in $Ca^{2+}$ flux across the presynaptic membrane [44]. A change in the level of norepinephrine in the synaptic cleft would lead to an opposing change in norepinephrine release. This concept was supported by the finding that the release of norepinephrine can be increased by α-blockers in concentrations which do not significantly block the membrane pump re-uptake mechanism [32, 40, 43, 45] nor affect the post-synaptic response to stimulation [39, 46]. It was also found that drugs which stimulated α-receptors caused a decrease in the amount of norepinephrine released per nerve impulse [39, 42, 46–48]. Thus, in the peripheral noradrenergic system it appears that the presynaptic receptor functions as a braking or damping mechanism, causing decreased release when levels of norepinephrine in the synapse are increased [49].

More recently it has become apparent that related phenomena may exist in the central nervous system. Field-stimulated cortical and striatal slices which have been preincubated with [$^3$H]norepinephrine or [$^3$H]dopamine show changes in the overflow of radioactivity when drugs known to affect noradrenergic or dopaminergic receptors are added to the incubating medium [50–53]. Whether the dopaminergic receptor mediating the changes in tyrosine hydroxylase activity described in this paper also has a role in modulating transmitter release remains to be established. However, the presynaptic receptor in the nigro-neostriatal dopamine system does appear to be capable of exerting a modulatory influence on the increase in tyrosine hydroxylase activity which occurs after inhibition of impulse flow.

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