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Acute transsynaptic regulation of tyrosine 3-monooxygenase activity in the rat superior cervical ganglion: Evidence for both cholinergic and noncholinergic mechanisms

(sympathetic ganglion/acetylcholine/potassium depolarization/cAMP/nerve stimulation)

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ABSTRACT **The rate of dopa synthesis in the rat superior cervical ganglion was increased 4- to 6-fold during continuous electrical stimulation of the cervical sympathetic trunk at 10 Hz for 30 min. This increase was only partially blocked by 3 mM hexamethonium and was not significantly affected by 6 μM atropine. In the presence of both hexamethonium and atropine, nerve stimulation still produced a 2- to 4-fold increase in dopa synthesis. Physostigmine increased dopa synthesis in both control and stimulated ganglia. This effect of physostigmine was completely blocked by hexamethonium and atropine. Dopa synthesis was also significantly increased when ganglia were incubated in a medium containing an elevated concentration of K^+ (55 mM). This stimulatory effect of high K^+ was totally dependent on the presence of Ca^{2+} in the medium, was decreased by 60% by prior decentralization of the ganglion, and was unaffected by hexamethonium and atropine. The data demonstrate that tyrosine hydroxylase activity is rapidly increased after preganglionic nerve stimulation and suggest that this increase is mediated in part by acetylcholine and in part by a second (noncholinergic) transmitter. The effects of an elevated K^+ concentration may be mediated both by the release of a noncholinergic transmitter from the preganglionic nerve terminals and by direct depolarization of the ganglionic neurons.**

The ability to block the postsynaptic consequences of presynaptic nerve stimulation with a specific pharmacological antagonist is a crucial piece of evidence in identifying which transmitter or transmitters act at a particular synapse. Thus, the finding in bullfrog paravertebral ganglia that nicotinic and muscarinic antagonists block most, but not all, of the postsynaptic electrophysiological changes produced by preganglionic nerve stimulation indicates that acetylcholine is not the sole preganglionic neurotransmitter in these ganglia (1).

Data presented in the present report indicate that a similar situation holds in the rat superior cervical ganglion (SCG). In this study we examined a postsynaptic biochemical consequence of preganglionic nerve stimulation—namely, the acute increase in the activity of tyrosine 3-monooxygenase (tyrosine hydroxylase; TyrOHase; EC 1.14.16.2). Regulation of this enzyme is of interest because it catalyzes the rate-limiting step in norepinephrine biosynthesis. We have shown previously that pharmacological activation of either nicotinic or muscarinic receptors in the SCG leads to a rapid increase in TyrOHase activity (2). We now report that preganglionic nerve stimulation also causes a rapid elevation of enzyme activity and that this increase is mediated in part via a cholinergic (nicotinic) mechanism and in part via a mechanism that is insensitive to both nicotinic and muscarinic antagonists. A preliminary report of this work has been presented (3).

METHODS

Adult male Sprague–Dawley rats (175–200 g) were housed in individual cages under controlled lighting (12 hr light:12 hr dark) with ad lib access to food and water for 1 wk prior to each experiment. The rats were killed by cervical dislocation. Both SCG were rapidly removed together with short lengths of their preganglionic (about 5 mm) and postganglionic (about 2 mm) trunks. The ganglia were desheathed and maintained *in vitro* at 37°C. For experiments involving nerve stimulation, ganglia were preincubated for 20 min in 2.5 ml of a bicarbonate-buffered medium (see below) equilibrated with 95% O_2/5% CO_2. When the effects of hexamethonium, atropine, or physostigmine were to be examined, these drugs were added to the medium 10 min before stimulation. Contralateral control ganglia from the same animals were maintained under similar conditions without stimulation. The preganglionic nerve trunk of one ganglion was stimulated at 10 Hz via a suction electrode, and the effectiveness of stimulation was monitored throughout the stimulation period by recording the compound action potentials in the postganglionic internal carotid nerve via a second suction electrode. Except where noted, the current intensity used was twice that required to give a maximal compound action potential.

TyrOHase activity was assayed by measuring the rate of accumulation of dopa in the presence of brocresine, an inhibitor of dopa decarboxylase (EC 4.1.1.28). We have shown previously that (*i*) ganglia incubated in the absence of brocresine contain no measurable dopa, (*ii*) dopa accumulation, in the presence of brocresine, is blocked by the TyrOHase inhibitor 3-iodotyrosine, and (*iii*) dopa is stable under our incubation conditions for at least 30 min (2). To initiate the measurement of dopa synthesis, brocresine (150 μM) was added to the incubation medium 2 min before stimulation was begun. At the end of the stimulation period, stimulated and control ganglia were each homogenized in 250 μl of 1.65 M trichloroacetic acid/1 mM EDTA. Incubation media were added to the respective homogenates, and the combined samples were chilled on ice and then centrifuged for 2 min in a Beckman Microfuge. The supernatants were stored at −20°C prior to determination of their dopa content. Dopa was measured by high-performance liquid chromatography (4).

For experiments concerned with the effects of K^+ and carbachol, ganglia were preincubated for 30 min in 650 μl of me-

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Abbreviations: TyrOHase, tyrosine hydroxylase; SCG, superior cervical ganglion.

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dium without brocresine prior to transfer to the appropriate brocresine-containing medium. For studies on K^+, NaCl was replaced with KCl to give a final K^+ concentration of 55 mM. When cholinergic antagonists were used, they were present during both preincubation and incubation periods. At the end of the incubation period, ganglia were homogenized in 65 μl of 1.65 M trichloroacetic acid/1 mM EDTA. The incubation media were added to the homogenates and the combined samples were treated as described above. In one experiment, ganglia were decentralized 4 days prior to the experiment in order to cause the preganglionic nerve terminals to degenerate (5). For this purpose, animals were anesthetized with chloral hydrate (640 mg/kg, subcutaneously), and a short section of each cervical sympathetic trunk was removed about 6 mm proximal to the ganglion. In this experiment, ganglia from sham-operated animals served as controls.

Two different media were used in these experiments. In the initial stimulation experiments (Figs. 1 and 2), Kreb's bicarbonate-buffered medium [136 mM NaCl/5.6 mM KCl/1.8 mM $CaCl_2$/1.2 mM $MgCl_2$/1.2 mM NaH_2PO_4/16.2 mM $NaHCO_3$/11.1 mM glucose (6)] was used and was supplemented with 0.2 mM tyrosine, 50 μM choline bromide, and 0.1 mM EDTA, the latter to stabilize any dopa that might be released into the medium. In all other experiments, Earle's balanced salt solution (GIBCO; 116.3 mM NaCl/5.4 mM KCl/1.8 mM $CaCl_2$/0.8 mM $MgSO_4$/1.0 mM NaH_2PO_4/26 mM $NaHCO_3$/5.5 mM glucose/28 μM phenol red) was used and was supplemented with 0.1 mM tyrosine and 0.1 mM EDTA. The rate of dopa synthesis in control ganglia differed significantly depending on the medium used. Ganglia incubated in the supplemented Kreb's solution synthesized dopa at about twice the rate of ganglia incubated in the supplemented Earle's balanced salt solution. This difference in the control rate of dopa synthesis was confirmed in a separate experiment in which the effects of the two media were compared directly (data not shown). In the same experiment, the rate of dopa synthesis in stimulated ganglia (10 Hz for 30 min) was independent of the medium used. We have not yet determined what component(s) of the media is responsible for the difference in the control rates.

Data are expressed as the mean rate (± SEM) of dopa synthesis (pmol per ganglion per hr) of three to six ganglia. The values for stimulated and control ganglia were compared by Student's paired *t* test (two-tailed). The significance of the differences between all other groups was assessed by the *t* test for two means (two-tailed, except where noted).

Chloral hydrate, carbachol, hexamethonium bromide, atropine sulfate, physostigmine sulfate, and 8-bromoadenosine 3',5'-cyclic monophosphate were purchased from Sigma. Brocresine was purchased from Smith and Nephew (U.K.).

RESULTS

The accumulation of dopa in both synaptically stimulated and control ganglia increased linearly with time of incubation up to at least 30 min (Fig. 1). Preganglionic nerve stimulation at 10 Hz resulted in about a 4-fold increase in the rate of dopa synthesis. Addition of the nicotinic antagonist hexamethonium to the incubation medium at 3 mM (a concentration we found sufficient to completely block the compound action potentials normally recorded from the postganglionic trunk) inhibited the increase in TyrOHase activity by 43% (Fig. 2). Thirty millimolar hexamethonium produced no further inhibition (data not shown). Addition of the muscarinic antagonist atropine at 6 μM [a concentration that is sufficient to block muscarinic electrophysiological responses in the SCG (7, 8)] did not significantly alter the increase in TyrOHase activity caused by nerve stimulation. In the presence of both 3 mM hexamethonium and 6 μM atropine, preganglionic nerve stimulation still produced about a 2.5-fold increase in dopa accumulation (Fig. 2). In fact, even when 10-fold higher concentrations of both cholinergic antagonists were used, an increase in dopa synthesis of about 2.5-fold was still observed (data not shown).

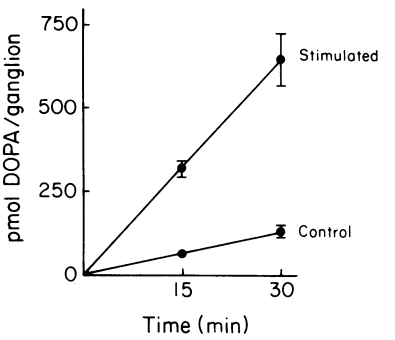

FIG. 1. Accumulation of dopa in stimulated and contralateral control ganglia. The SCG from one side of the animal was stimulated via the preganglionic nerve for 15 or 30 min at 10 Hz. The contralateral control ganglion was maintained under similar *in vitro* conditions without stimulation. Dopa synthesis was measured during the stimulation period (see *Methods*). Each point represents the mean ± SEM of three or four ganglia.

In an attempt to provide further evidence as to whether the hexamethonium and atropine-resistant stimulation of dopa synthesis involved acetylcholine, ganglia were stimulated in the presence of the acetylcholinesterase inhibitor physostigmine. Addition of physostigmine (20 μM) to the incubation medium significantly elevated the rate of dopa synthesis in both control and stimulated ganglia (Fig. 3). However, these effects of physostigmine were completely blocked by addition of hexamethonium and atropine. Thus, the magnitude of the hexamethonium and atropine-resistant increase in dopa synthesis produced by preganglionic nerve stimulation was not altered by physostigmine. Physostigmine also led to a rapid decrement in the size of the compound action potentials recorded from the postganglionic internal carotid nerve during preganglionic stimulation at 10 Hz. Within 1 min of the onset of stimulation, the compound action potentials were decreased by about 90%.

In addition to examining the effects of preganglionic nerve

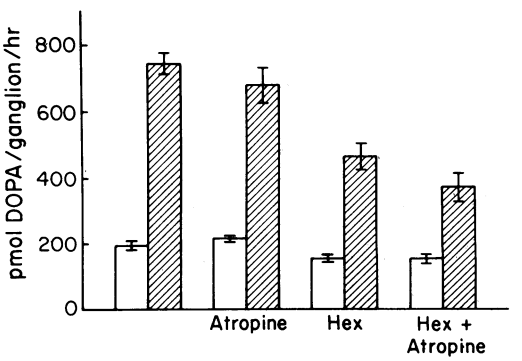

FIG. 2. Effect of cholinergic antagonists on the increase in dopa production during preganglionic nerve stimulation. Ganglia were stimulated at 10 Hz for 30 min. Three millimolar hexamethonium (Hex) and 6 μM atropine were added 10 min prior to the beginning of stimulation. Data for the atropine and the Hex with atropine groups are the means ± SEM of three ganglia. The other groups each contained seven ganglia. Stimulated ganglia, hatched bars; control ganglia, open bars.

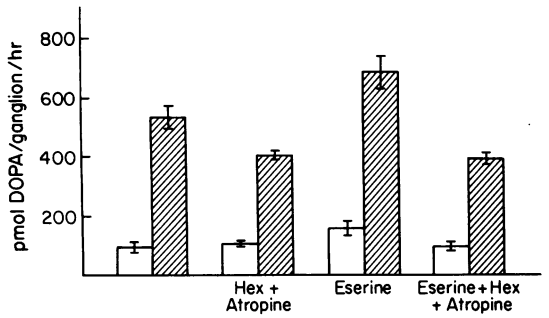

FIG. 3. Effects of physostigmine (eserine) and cholinergic antagonists on the increase in dopa production during preganglionic nerve stimulation. The current intensity used was that required to give a maximal compound action potential. Ganglia were stimulated (hatched bars) at 10 Hz for 30 min. Twenty micromolar eserine, 3 mM hexamethonium (Hex), and 6 μM atropine were added 10 min before stimulation was begun. Each bar represents the mean ± SEM of three or four ganglia. Eserine significantly increased the rate of dopa synthesis in both control (open bars) and stimulated ganglia ($P < 0.05$ by one-tailed t test for both comparisons).

stimulation, we have investigated the consequences of incubating ganglia in medium containing an elevated concentration of K^+. When ganglia were incubated in medium containing 55 mM K^+, dopa synthesis was elevated 7- to 9-fold (Fig. 4). This effect of high K^+ was decreased by 60% in ganglia that had been decentralized 4 days earlier, although decentralization had no significant effect on the stimulation of dopa synthesis produced by carbachol (0.1 mM) (Fig. 4). The effect of high K^+ was totally abolished when normal ganglia were incubated in a medium lacking Ca^{2+} and containing 0.1 mM EGTA (Table 1). Addition of 3 mM hexamethonium and 6 μM atropine did not affect the stimulation of dopa synthesis produced by high K^+, although these antagonists totally blocked the response to carbachol (Fig. 5). Even 10-fold higher concentrations of hexamethonium and atropine did not decrease the effect of high K^+ (data not shown).

Analogues of cAMP have been found to elevate TyrOHase activity acutely in a variety of neural systems (e.g., see refs. 9 and 10). Therefore, we examined the effects of 8-bromoadenosine 3',5'-cyclic monophosphate on ganglionic TyrOHase. At a concentration of 0.5 mM the cyclic nucleotide elevated the rate of dopa synthesis by about 40% (106 ± 6 compared to 77 ± 3 pmol per ganglion per hr).

DISCUSSION

We recently reported that incubation of the SCG with nicotinic and muscarinic agonists leads to an acute stimulation of TyrOHase activity (2). The maximal effect achieved by nicotinic stimulation using dimethylphenylpiperazinium was 4-fold, whereas the maximal effect resulting from muscarinic stimulation using bethanechol was 2-fold. In the current study, we examined whether these cholinergic mechanisms were activated by preganglionic nerve stimulation and found that TyrOHase activity was increased acutely in part via a nicotinic mechanism. No evidence was observed for a muscarinic component in this increase; however, it remains possible that an effect of muscarinic transmission would be detectable under certain conditions known to facilitate muscarinic responses in the SCG, such as stimulation with higher frequencies (8) or stimulation in the presence of an acetylcholinesterase inhibitor (11). The experiment performed with physostigmine (Fig. 3) does not allow one to evaluate the latter possibility because both nicotinic and muscarinic antagonists were present in this experiment.

A quite unexpected finding of the present study was the observation that a large proportion of the acute increase in TyrOHase activity caused by preganglionic nerve stimulation could not be blocked by adding both nicotinic and muscarinic antagonists to the medium. Thus, addition of both 3 mM hexamethonium and 6 μM atropine only blocked the increase in TyrOHase activity by 30–50%, although the compound action potentials recorded from the postganglionic trunks were completely abolished. It is possible that nerve stimulation might result in some cholinergically evoked subthreshold depolarizations even in the presence of these concentrations of cholinergic antagonists and that these cholinergic responses could mediate the residual increase in TyrOHase activity. However, this seems unlikely due to the finding that increasing the concentration of the cholinergic antagonists 10-fold failed to cause any further decrease in the biochemical response. Thus, the data suggest that the residual increase in TyrOHase activity in the presence of hexamethonium and atropine is mediated

Table 1. Stimulation of dopa synthesis by K^+ depends on the presence of Ca^{2+} in the medium

Medium	Dopa synthesis, pmol/ganglion/hr	
	Control	55 mM K^+
Complete	70 ± 11	663 ± 40
Ca^{2+}-free*	47 ± 10	59 ± 4

Ganglia were preincubated in complete medium or Ca^{2+}-free medium for 30 min and then were incubated in these media in the presence or absence of elevated K^+ for 60 min. Data represent the means ± SEM of groups of three ganglia.
* Prepared by omitting $CaCl_2$ and including 0.1 mM EGTA.

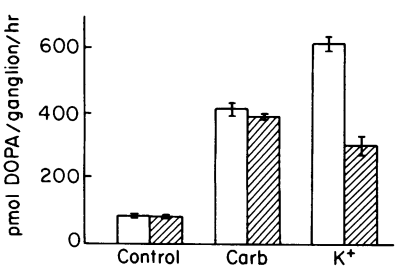

FIG. 4. Effects of carbachol and K^+ in sham-operated and decentralized ganglia. Groups of ganglia were decentralized 4 days prior to the experiment. Both sham-operated (open bars) and decentralized (hatched bars) ganglia were exposed to 0.1 mM carbachol (Carb) or 55 mM K^+ for 30 min. Each bar represents the mean ± SEM of four or five ganglia.

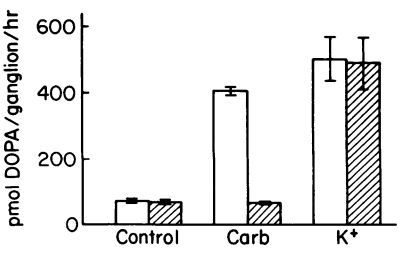

FIG. 5. Effects of cholinergic antagonists on the stimulation of dopa synthesis by carbachol and elevated K^+. Ganglia were incubated in medium containing either 0.1 mM carbachol (Carb) or 55 mM K^+ for 1 hr. Three millimolar hexamethonium (Hex) and 6 μM atropine were present in both preincubation and incubation media. Each bar represents the mean ± SEM of three ganglia. Hex and atropine, hatched bars; no additions, open bars.

by a noncholinergic transmitter. Further evidence for the involvement of a noncholinergic transmitter is the finding that although physostigmine increased the hexamethonium and atropine-sensitive component of TyrOHase regulation it did not affect the hexamethonium and atropine-resistant component. One could argue that the hexamethonium and atropine-resistant component may have been maximally activated in this experiment prior to addition of physostigmine. However, we have recently found that even under stimulation conditions in which this component is clearly not maximally activated physostigmine does not affect its magnitude.

Steinberg and Keller (12) hypothesized previously that preganglionic nerve stimulation causes an increase in norepinephrine synthesis in the rat SCG via a nonnicotinic, nonmuscarinic but cholinergic mechanism. They based this hypothesis on their findings that the increased rate of synthesis of [^{3}H]norepinephrine from [^{3}H]tyrosine caused by preganglionic nerve stimulation was not blocked by hexamethonium or atropine but was potentiated by physostigmine. However, they did not determine whether this effect of physostigmine could be prevented by hexamethonium and atropine. Our finding that the effect of physostigmine on dopa synthesis can be completely blocked by addition of nicotinic and muscarinic antagonists argues against the involvement of a novel cholinergic receptor in this system.

In addition to increasing as a result of preganglionic nerve stimulation, dopa synthesis was elevated when the K^+ concentration of the medium was raised to 55 mM. This increase in dopa synthesis was totally dependent on the presence of Ca^{2+} in the medium, was decreased after degeneration of the preganglionic nerve terminals, and was unaffected by high concentrations of hexamethonium and atropine. The calcium dependence of the high K^+ effect could indicate that direct depolarization of the ganglionic neurons leads to a stimulation of TyrOHase via an increased calcium influx, as has been hypothesized to occur in adrenergic nerve terminals (e.g., see ref. 13) and in pheochromocytoma cells (10), or it could reflect a requirement for transmitter release, or both. Our studies with decentralized ganglia suggest that in the intact SCG, K^+ increases dopa synthesis by a combination of both mechanisms. Thus, the fact that decentralization of the ganglion 4 days prior to an experiment decreased the effect of K^+ is consistent with one site of potassium's action being presynaptic. The effect of K^+ that remains after decentralization probably reflects a direct action on the ganglionic neurons. However, it should be noted that other interpretations cannot be excluded. For example, decentralization might decrease the responsiveness of the ganglionic neurons to the direct action of K^+, though no change in responsiveness to carbachol was seen.

If K^+ acts in part via release of transmitter(s) from the preganglionic nerve terminals, it is perhaps surprising that high concentrations of hexamethonium and atropine produce no inhibition of the response to K^+, particularly because these drugs significantly decrease the effect of preganglionic nerve stimulation. There are a number of possible explanations for this finding. For example, although high K^+ has been shown to release acetylcholine in the SCG (14), it may do so in amounts or with a time course that is inadequate for stimulation of dopa synthesis. Alternatively, if the mechanism by which cholinergic agonists stimulate dopa synthesis is by depolarization of ganglionic neurons, K^+ may both release acetylcholine and mimic the consequences of cholinergic receptor stimulation. Finally, K^+ may stimulate dopa synthesis supramaximally via a number of independent mechanisms (e.g., release of acetylcholine, release of a noncholinergic transmitter, and direct depolarization of the ganglionic neurons) such that removal of the cholinergic component still allows for a maximal stimulation.

In certain respects our data on the short-term regulation of TyrOHase activity resemble the findings of Quenzer *et al.* (15) on the regulation of cGMP in the rat SCG. These authors found that high K^+ and preganglionic nerve stimulation led to an increase in cGMP that was not blocked by cholinergic antagonists but was sensitive to decentralization. They suggested that either the increases in cGMP occurred in preganglionic nerve terminals or that they occurred in the ganglionic neurons and were stimulated by the release of a transmitter other than acetylcholine. This uncertainty results from the fact that cGMP, unlike TyrOHase, is likely to be present in both pre- and postsynaptic elements. If the increase in cGMP does occur in ganglionic neurons, it is possible that the same noncholinergic substance released by preganglionic nerve terminals might increase both cGMP and TyrOHase activity. However, cGMP is not likely to be a second messenger mediating the noncholinergic stimulation of TH activity because Ikeno *et al.* (16) and J. Horwitz (personal communication) found no effect on TyrOHase activity of incubating ganglia with derivatives of cGMP. cAMP concentrations may also be regulated by a noncholinergic, transsynaptic mechanism in the rat SCG. Evidence in favor of this idea includes the findings that cAMP concentrations in this ganglion can be increased by nerve stimulation or elevated K^+ but not by dimethylphenylpiperazinium or bethanechol (17–19). These results, together with our findings and those of Ikeno *et al.* (16) that derivatives of cAMP increase dopa synthesis, raise the possibility that cAMP could be an intracellular mediator of the noncholinergic stimulation of TyrOHase.

There are many possible candidates for the noncholinergic transmitter involved in TyrOHase regulation. For example, much attention has focused recently on the possibility that certain peptides may function as neurotransmitters in specific autonomic ganglia (20). In particular, in the bullfrog paravertebral ganglion, a "luteinizing hormone-releasing hormone-like" peptide has been shown to be present in preganglionic nerve terminals and to be released by preganglionic nerve stimulation (21). This peptide causes a depolarization of the ganglionic neurons that appears to mimic the noncholinergic late slow excitatory postsynaptic potential produced by preganglionic nerve stimulation (22). We have recently screened a large number of neuropeptides for their ability to increase TyrOHase activity in the rat SCG. Secretin and vasoactive intestinal peptide were found to increase TH activity about 3-fold even in the presence of both nicotinic and muscarinic antagonists (23). Further investigation is necessary to determine whether one or both of these peptides is involved in the noncholinergic regulation of TH activity after preganglionic nerve stimulation.

Note Added in Proof. Volle and Patterson (24) have reported, in a paper which appeared after this manuscript was submitted, that vasoactive intestinal peptide increases cAMP levels in the rat SCG.

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1. Nishi, S. & Koketsu, K. (1968) *J. Neurophysiol.* **31**, 109–121.
2. Ip, N. Y., Perlman, R. L. & Zigmond, R. E. (1982) *J. Pharmacol. Exp. Ther.* **223**, 280–283.
3. Ip, N. Y., Perlman, R. L. & Zigmond, R. E. (1982) *Trans. Am. Soc. Neurochem.* **13**, 106 (abstr.).
4. Erny, R. E., Berezo, M. W. & Perlman, R. L. (1981) *J. Biol. Chem.* **256**, 1335–1339.
5. Raisman, G., Field, P. M., Ostberg, A. J. C., Iversen, L. L. & Zigmond, R. E. (1974) *Brain Res.* **71**, 1–16.
6. Larrabee, M. G. & Bronk, D. W. (1952) *Cold Spring Harbor Symp. Quant. Biol.* **17**, 245–266.

7. Libet, B. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29,** 1945–1956.
8. McIsaac, R. J. (1977) *J. Pharmacol. Exp. Ther.* **200,** 107–116.
9. Weiner, N., Lee, F., Barnes, E. & Dreyer, E. (1977) in *Structure and Function of Monoamine Enzymes,* eds. Usdin, E., Weiner, N. & Youdim, M. B. H. (Dekker, New York), pp. 109–148.
10. Chalfie, M., Settipani, L. & Perlman, R. L. (1979) *Mol. Pharmacol.* **15,** 263–270.
11. Volle, R. L. (1980) in *Handbook of Experimental Pharmacology,* Pharmacology of Ganglionic Transmission, eds. Kharkevich, D. A. (Springer, Berlin), Vol. 53, pp. 385–410.
12. Steinberg, M. I. & Keller, C. E. (1978) *J. Pharmacol. Exp. Ther.* **204,** 384–399.
13. Weiner, N. & Lee, F.-L. (1975) in *Chemical Tools in Catecholamine Research,* eds. Almgren, O., Carlsson, A. & Engel, J. (North Holland, Amsterdam), pp. 61–71.
14. Collier, B. (1969) *J. Physiol. (London)* **205,** 341–352.
15. Quenzer, L. F., Patterson, B. A. & Volle, R. L. (1980) *J. Pharmacol. Exp. Ther.* **215,** 297–303.
16. Ikeno, T., Dickens, G., Lloyd, T. & Guroff, G. (1981) *J. Neurochem.* **36,** 1632–1640.
17. Aleman, V., Bayou, A. & Molina, J. (1974) *Adv. Behav. Biol.* **10,** 115–124.
18. Kalix, P. & Roch, P. (1976) *Gen. Pharmacol.* **7,** 267–270.
19. Quenzer, L., Yahn, D., Alkadhi, K. & Volle, R. L. (1979) *J. Pharmacol. Exp. Ther.* **208,** 31–36.
20. Burnstock, G., Hökfelt, T., Gershon, M. D., Iversen, L. L., Kosterlitz, H. W. & Szurszewski, J. H. (1978) *Neurosci. Res. Program, Bull.* **17,** 424–443.
21. Jan, Y. N., Jan, L. Y. & Kuffler, S. W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1501–1505.
22. Jan, Y. N., Jan, L. Y. & Kuffler, S. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5008–5012.
23. Ip, N. Y., Ho, C. K. & Zigmond, R. E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7566–7569.
24. Volle, R. L. & Patterson, B. A. (1982) *J. Neurochem.* **39,** 1195–1197.