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REFERENCES

1. M. S. Amer and W. E. Kreighbaum, *J. Pharmac. Sci.* **64**, 1 (1975).
2. B. Weiss and W. N. Hait, *A. Rev. Pharmac. Toxicol.* **17**, 441 (1977).
3. G. Nemoz, A. F. Prigent and H. Pacheco, *Biochem. Pharmac.* **27**, 2769 (1978).
4. A. F. Prigent, A. Grouiller, H. Pacheco and A. Cier, *Chim. Ther.* **7**, 329 (1972).
5. A. F. Prigent, A. Grouiller and H. Pacheco, *Eur. J. Med. Chem.* **10**, 490 (1975).
6. G. Brooker, L. J. Thomas, Jr. and M. M. Appleman, *Biochemistry* **7**, 4177 (1968).
7. W. J. Thompson and M. M. Appleman, *Biochemistry* **10**, 311 (1971).
8. R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam (1977).
9. C. Hansch, A. Leo, S. M. Unger, K. M. Kim, D. Nikaitani and E. J. Lien, *J. Med. Chem.* **16**, 1207 (1973).
10. A. Verloop, W. Hoogenstraaten and J. Tipker, *Med. Chem. (Academic)* **11**, 165 (1976).
11. V. Stefanovich, M. Von Polnitz and M. Reiser, *Arzneimittel Forsch.* **24**, 1747 (1974).
12. W. J. Thompson and M. M. Appleman, *J. biol. Chem.* **246**, 3145 (1971).
13. R. W. Butcher and E. W. Sutherland, *J. biol. Chem.* **237**, 1244 (1962).
14. E. B. Goodsell, H. H. Stein and K. J. Wenzke, *J. Med. Chem.* **14**, 1202 (1971).
15. J. E. Garst, G. L. Kramer, Y. J. Wu and J. N. Wells, *J. Med. Chem.* **19**, 499 (1976).
16. G. L. Kramer, J. E. Garst, S. S. Mitchel and J. N. Wells, *Biochemistry* **16**, 3316 (1977).

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3,4-Dihydroxyphenylacetic acid content of sympathetic ganglia as a possible biochemical indicator of small intensely fluorescent cell participation in ganglionic transmission

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Dopamine (DA) in the small intensely fluorescent (SIF) cells of sympathetic ganglia [1], is thought to be released onto sympathetic neurons as a result of stimulation of muscarinic receptors on the SIF cells by preganglionic cholinergic neurons [2]. The concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) within the ganglia is a relative measure of DA formation and metabolism [3]. Presently there is no biochemical technique to evaluate sympathetic ganglionic transmission in freely moving rats. It may be possible, however, to monitor the participation of the SIF cells in ganglionic transmission by analyzing the content of DOPAC in the ganglion. In a model proposed by Libet [4], DA released from ganglionic SIF cells in response to preganglionic cholinergic neuronal activation might produce at least three actions: (a) elicit a direct hyperpolarization of the principal neurons; (b) produce a long-lasting modulatory change in the muscarinic response of the principal neurons to acetylcholine; and (c) reduce the release of acetylcholine from preganglionic neurons. In this report we demonstrate that drugs that interact with cholinergic, adrenergic and dopaminergic receptors alter the metabolism of DA in the celiac ganglion of the rat. The celiac ganglion was chosen for study because it apparently has the highest rate of DA metabolism of the rat sympathetic ganglia [5].

DA and DOPAC were analyzed in a single celiac ganglion by gas chromatography–mass spectrometry, as described previously [3]. The doses of the drugs and the time of killing are shown in Table 1. Our previous studies have shown that the doses of the drugs administered altered brain biogenic amine metabolism. Celiac ganglia were removed under a dissecting microscope after rats (male, Sprague–Dawley, 150–180 g, obtained from Zivic Miller Laboratories, Allison Parks, PA) were decapitated.

The content of DA in the ganglion remained essentially normal after each of the drug treatments (Table 1). In contrast, DOPAC content changed in a manner which suggested that the drugs had activated or inhibited specific receptors. As reported previously, treatment with the muscarinic agonist oxotremorine induced a rise of DOPAC in the ganglion, a rise that was antagonized by atropine pretreatment [5]. Atropine treatment alone resulted in a fall of DOPAC content, suggesting that normally there is tonic activation of SIF cell muscarinic receptors by preganglionic cholinergic neurons.

The β-adrenergic receptor agonist isoproterenol had no effect on the content of DOPAC in the celiac ganglion. In contrast, the α-adrenergic receptor agonist phenylephrine decreased the content of DOPAC, and prior treatment with phenoxybenzamine prevented the fall of DOPAC. Moreover, phenylephrine could partially antagonize the rise of DOPAC after oxotremorine treatment.

The administration of the DA agonist apomorphine had not effect on the content of DOPAC, but the DA antagonist haloperidol, administered alone, significantly increased DOPAC. The increase was not blocked by administering haloperidol together with apomorphine, but it appeared to be blocked by treatment with atropine. The apparent blockade, however, may be the algebraic sum of the fall normally induced by atropine and the rise induced by haloperidol. Phenylephrine treatment reversed the elevated levels of DOPAC induced by haloperidol as well. The blockade of the haloperidol-induced rise of DOPAC by atropine and phenylephrine probably represents physiological antagonism rather than competitive blockade at a common receptor. Whether the haloperidol-induced increase of DOPAC in the ganglia is the consequence of release of DA metabolism from the constraints of a negative

Table 1. Modulation of dopamine metabolism in the celiac ganglion of the rat by receptor agonists and antagonists*

Treatment	Dopamine (nmoles/mg protein ± S.E.M.)	DOPAC (nmoles/mg protein ± S.E.M.)
I Saline	24 ± 3	26 ± 2
Oxotremorine	25 ± 2	73 ± 5†
Atropine	17 ± 3	16 ± 2†
Atropine + oxotremorine	24 ± 3	25 ± 3
Oxotremorine + phenylephrine	22 ± 5	50 ± 4†‡
II Saline	22 ± 3	25 ± 2
Isoproterenol	26 ± 4	29 ± 3
Phenylephrine	18 ± 3	14 ± 2†
Phenoxybenzamine	19 ± 3	23 ± 2
Phenoxybenzamine + phenylephrine	18 ± 3	24 ± 1
III Saline	23 ± 4	26 ± 2
Apomorphine	30 ± 5	25 ± 1
Haloperidol	25 ± 6	38 ± 4†
Haloperidol + apomorphine	31 ± 5	49 ± 5†
Atropine + haloperidol	17 ± 4	24 ± 2
Haloperidol + phenylephrine	17 ± 2	6 ± 1†

* Drug dosage and min to killing: oxotremorine sesquifumarate, 0.2 mg/kg, s.c., 15 min; phenylephrine HCl, 10 mg/kg, s.c., 10 min; ± isoproterenol HCl, 5 mg/kg, s.c., 20 min; apomorphine HCl, 5 mg/kg, i.p., 45 min; haloperidol, 1 mg/kg, i.p., 90 min; phenoxybenzamine HCl, 20 mg/kg, i.p., 60 min; and atropine sulfate, 20 mg/kg, i.p., 120 min. N = 5–10.

† $P < 0.05$, when compared with saline-treated rats.

‡ $P < 0.05$, when compared with oxotremorine-treated rats.

neuronal feedback loop in the ganglia, as has been postulated for striatum [6], remains to be investigated. Sympathetic ganglia may, indeed, be simple model systems for investigating negative neuronal feedback loops.

Our finding that phenylephrine reduced DA metabolism suggests a model for further study. Perhaps norepinephrine released from the cell bodies or dendrites of sympathetic neurons onto SIF cells inhibits DA release and metabolism by acting on α-adrenergic receptors. The inhibitory α-adrenergic receptor appears to be rather efficient at antagonizing the increase of DA metabolism induced by activating muscarinic receptors as well as that induced by administering haloperidol. A reciprocal system could serve to modulate information transfer between SIF cells and sympathetic neurons.

In our study, the drugs were administered to the rats and the metabolism of DA was evaluated thereafter. Thus, the drugs might interact with receptors on SIF cells or receptors associated with other peripheral and/or central structures, consequently altering sympathetic ganglionic transmission. Muscarinic receptors were shown to be associated with SIF cells of sympathetic ganglia by electrophysiological [2] and pharmacological techniques [3]. There is also evidence for nicotinic receptors playing a role in SIF cell DA metabolism [3].

The model proposed by Libet [4] predicts that ganglionic transmission would be altered by the release of DA from SIF cells onto principal neurons. Our studies suggest that DA acting via a negative neuronal feedback loop and norepinephrine released from the principal neurons might alter the release and metabolism of DA by the SIF cells. Moreover, they suggest that drugs that alter DOPAC content in ganglia might be identified for further study on ganglionic transmission. These drugs might interact with receptors on the SIF cells or at sites other than the ganglia to alter transmission.

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REFERENCES

1. A. Björklund, L. Cegrell, B. Falck, M. Ritzén and E. Rosengren, *Acta physiol. scand.* **78**, 334 (1970).
2. B. Libet and C. Owman, *J. Physiol., Lond.* **237**, 635 (1974).
3. F. Karoum, C. K. Garrison, N. H. Neff and R. J. Wyatt, *J. Pharmac. exp. Ther.* **201**, 654 (1977).
4. B. Libet, in *Advances in Biochemical Psychopharmacology* (Eds. E. Costa and G. L. Gessa), Vol. 16, p. 541. Raven Press, New York (1977).
5. B. E. Lutold, F. Karoum and N. H. Neff, *Eur. J. Pharmac.* **54**, 21 (1979).
6. N.-E. Andén, *J. Pharm. Pharmac.* **24**, 905 (1972).