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INFLUENCE OF SEROTONIN ON ADRENERGIC MECHANISMS

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Abstract—The influence of serotonin on the accumulation and metabolism of [^{3}H]norepinephrine by isolated perfused rat hearts has been investigated. Serotonin, at a concentration of 10—^{6} M, inhibits the uptake of [^{3}H]norepinephrine. A serotonin concentration of 10^{-4} M appears to reduce the neuronal norepinephrine storage capacity as well as the neuronal membrane amine transport system. Serotonin (10^{-5} M) also inhibits the extraneuronal metabolism of [^{3}H]norepinephrine in cocainized hearts similar to adrenergic receptor blockers. It is concluded that serotonin at low concentrations would enhance adrenergic responses by blocking the uptake of norepinephrine, while high concentrations of serotonin would block adrenergic receptors and tissue response to norepinephrine. Diminished neuronal amine storage in the presence of serotonin may lead to a prolonged decrease in response to adrenergic nerve stimulation due to a diminished release of authentic transmitter.

BIOGENIC amines are widely distributed throughout most living organisms, and evidence indicates that these amines function as neurohormones, either activating the post-synaptic membrane or modifying the action of a transmitter substance. The fact that serotonin and norepinephrine act as antagonists in many experimental conditions has led to the possibility that specific behavioral patterns or physiological actions may be influenced by the interaction of these two amines.[1–3]

Norepinephrine, injected into the circulation or released from nerve endings during stimulation, is rapidly inactivated either by uptake into sympathetic nerve endings or by metabolism at non-neuronal sites.[4] Norepinephrine accumulation and metabolism in the heart have been demonstrated many times.[5,6] This study demonstrates the influence of serotonin on [^{3}H]norepinephrine uptake, storage and metabolism in the isolated perfused rat heart.

METHODS

Male Royal Hart rats (175–200 g) were anesthetized with sodium pentobarbital, 30 mg/kg. Hearts were removed and perfused by the Langendorff technique as previously described.[7]

The drugs used were D,L-norepinephrine hydrochloride (Sigma Chemical Company), D,L-norepinephrine-7-^{3}H (New England Nuclear; specific activity, 6·6 c/mmole), 5-hydroxytryptamine creatine sulfate complex (Sigma Chemical Company) and cocaine hydrochloride (Merck).

Hearts were perfused for 10 min with norepinephrine-free medium to wash out blood and allow rhythmic beating to be established. This was followed by a 2-min perfusion with [^{3}H]norepinephrine (50 μc/l.) containing medium. Perfusion for 2 min is known to provide amine accumulation values close to initial rates of uptake.[8]

The hearts were then perfused another 2 min with norepinephrine-free medium to wash out extracellular [^{3}H]norepinephrine.[9] During the last 4 min of perfusion, the heart perfusates were collected in beakers and stored frozen for metabolite analysis.

The perfusion procedure was repeated with serotonin in both media at concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M.

To study the effect of serotonin on the non-neuronal metabolism of [^{3}H]norepinephrine, cocaine (10^{-4} M) was added to both perfusion media and the procedures described above were repeated.

After perfusion, the hearts were weighed, homogenized in 5 ml of 0·4 N perchloric acid and centrifuged for 10 min at 500 *g*. Aliquots (4 ml) of the heart supernatants and all of the collected perfusates were assayed for [^{3}H]norepinephrine and its metabolites as described by Kopin *et al.*[10]

The accumulation of [^{3}H]norepinephrine by isolated hearts in the presence of serotonin was determined by perfusing the hearts for various time intervals (5–40 min with [^{3}H]norepinephrine, 50 μc/l.; specific activity, 5 mc/μmole). Serotonin (10^{-4} M) was included in both media. Perfusion times are listed in Table 1. The hearts were perfused with Krebs–Ringer buffer for 2 min after the norepinephrine perfusion.

Aliquots (1 ml) of the heart supernatants were assayed for tritium in a liquid scintillation counter and the tritium was calculated as [^{3}H]norepinephrine. It is known that greater than 95 per cent of the tritium in hearts perfused with [^{3}H]-norepinephrine is associated with norepinephrine.[11]

TABLE 1. PERFUSION TIMES UTILIZED IN DETERMINING THE EFFECTS OF SEROTONIN ON THE ACCUMULATION OF NOREPINEPHRINE BY THE ISOLATED RAT HEART*

Krebs–Ringer (min)	Krebs–Ringer with norepinephrine (min)
19·0	2
17·5	5
15·0	10
10·0	20
5·0	30
0	40

* Serotonin was included in both perfusion media. A 2-min perfusion of Krebs–Ringer followed the norepinephrine perfusion.

The influence of serotonin on soluble catechol-*O*-methyltransferase (COMT) activity was determined by modifying a method described previously by Axelrod *et al.*[12] Rats were treated with the monoamine oxidase inhibitor pargyline (25 mg/kg, intraperitoneally) 4 hr prior to sacrifice. Hearts were removed and homogenized in 5 vol. of Krebs–Ringer buffer (with bicarbonate) containing 3·6 M magnesium sulfate. The heart homogenates were centrifuged for 20 min at 14,000 *g*. The tissue and tissue extracts were maintained at 5° at all times until the incubation period. Krebs–Ringer buffer (75 μl) which contained 0·4 μM magnesium sulfate, 70 mμc [^{3}H]-norepinephrine (specific activity, 2·3 c/μmole) and 60 mμmoles S-adenosylmethionine per 75-μl aliquot was added to 25 μl of the heart supernatant and incubated for

90 min at 37°. Blanks were obtained by substituting boiled tissue extract. The reaction was terminated by the addition of 0·5 ml of 0·4 N perchloric acid. [^{3}H]Normetanephrine was assayed as indicated above.

RESULTS

[^{3}H]Norepinephrine uptake is inversely proportional to the perfusate concentration of serotonin (Fig. 1). When perfused at 10^{-5} M, serotonin inhibited the accumulation of [^{3}H]norepinephrine by 43 per cent. At higher concentrations of serotonin (10^{-4} and 10^{-3} M), the uptake of [^{3}H]norepinephrine was reduced by 75 and 88 per cent respectively.

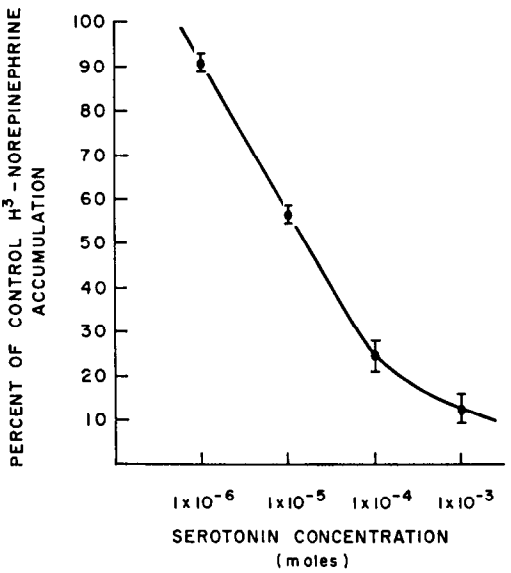

FIG. 1. Effect of serotonin on [^{3}H]norepinephrine accumulation in the isolated perfused rat heart Hearts were perfused for 10 min with or without serotonin followed by a 2-min perfusion with [^{3}H]norepinephrine. The perfusions were concluded with a 2-min washout of norepinephrine-free media. Each value is the mean ± S.E.M. of at least eight hearts.

Iversen[5] has suggested that the accumulation of norepinephrine during short perfusion periods represents the rate of membrane transport, while amine accumulation during long perfusions is indicative of intraneuronal storage capacity. Serotonin (10^{-4} M) greatly reduced the accumulation of [^{3}H]norepinephrine at all perfusion times (Fig. 2). Thus, serotonin appears to interfere not only with the neuronal membrane transport, but also with neuronal storage.

The effects of serotonin on the metabolism of [^{3}H]norepinephrine by the rat heart are illustrated in Table 2. At 10^{-5} M, serotonin reduced [^{3}H]norepinephrine uptake by the heart tissue more than 40 per cent. However, at this concentration, serotonin did not influence [^{3}H]norepinephrine metabolism. At 10^{-4} M, serotonin reduced [^{3}H]norepinephrine uptake in the heart 75 per cent with a 22 per cent reduction in [^{3}H]normetanephrine formation and a 30 per cent decrease in [^{3}H] deaminated catechols.

To study the influence of serotonin on the extraneuronal fate of norepinephrine,

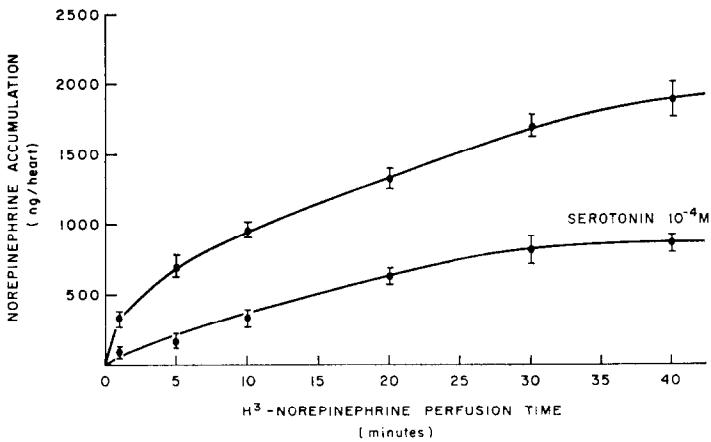

FIG. 2. Influence of serotonin on norepinephrine accumulation in the isolated perfused rat heart. Perfusion times are listed in Table 1. The [^{3}H]norepinephrine perfusate contained 200 ng/ml of norepinephrine. Each value is the mean ± S.E.M. of at least eight hearts.

TABLE 2. EFFECT OF SEROTONIN ON THE METABOLITES OF NOREPINEPHRINE IN THE ISOLATED PERFUSED RAT HEART*

[^{3}H]-metabolites	Control (mμc ^{3}H/ heart)	Serotonin (10^{-5} M) (mμc ^{3}H/ heart)	Change (%)	P	Serotonin (10^{-4}M$^-$ (mμc ^{3}H/ heart)	Change (%)	P
Normetanephrine	9·35 ± 0·32	9·20 ± 0·42	−1·57	N.S.	7·24 ± 0·50	−22·57	<0·005
Deaminated catechols	12·53 ± 0·97	13·40 ± 0·52	6·88	N.S.	8·73 ± 0·41	−30·32	<0·001

* Hearts were perfused for 10 min with or without serotonin, followed by a 2-min perfusion of [^{3}H]norepinephrine. The perfusions were concluded with a 2-min washout of norepinephrine-free media. Values are the mean ± S.E.M. of at least six hearts and represent the total of each metabolite in tissue and perfusate. N.S. = not significant.

TABLE 3. EFFECT OF SEROTONIN ON THE UPTAKE AND METABOLISM OF NOREPINEPHRINE IN THE COCAINIZED RAT HEART*

[^{3}H]-metabolites	Control (mμc ^{3}H/ heart)	Serotonin (10^{-5} M) (mμc ^{3}H/ heart)	Change (%)	P	Serotonin (10^{-4} M) (mμc ^{3}H/ heart)	Change (%)	P
Norepinephrine	47·32 ± 4·59	38·90 ± 2·75	−17·79	N.S.	20·10 ± 1·92	−57·51	<0·001
Normetanephrine	22·78 ± 1·34	15·84 ± 0·44	−30·48	<0·005	10·77 ± 0·83	−52·73	<0·001
Deaminated catechols	33·11 ± 4·35	24·27 ± 1·34	−26·69	N.S.	14·35 ± 0·15	−56·60	<0·005

* Hearts were perfused as described under Table 2, except that cocaine (10^{-4} M) was included in all perfusion media. Values are the mean ± S.E.M. of at least six hearts and represent the total of each metabolite in tissue and perfusate (except for norepinephrine, which only includes tissue levels).

cocaine (10^{-4} M) was included in all perfusion media. Under these conditions, serotonin (10^{-5} M) reduced [^{3}H]norepinephrine in the heart 17 per cent, [^{3}H]normetanephrine 30 per cent and the H-deaminated catechols by 26 per cent. At 10^{-4} M, serotonin reduced [^{3}H]norepinephrine, [^{3}H]normetanephrine and [^{3}H]deaminated catechols by 57, 52 and 56 per cent respectively (Table 3).

Serotonin (10^{-4} M) incubated with soluble COMT did not reduce the activity of this enzyme.

DISCUSSION

Various investigations indicate that serotonin can either enhance or inhibit the the action of norepinephrine. Scroop and Walsh[13] have demonstrated that serotonin potentiates the norepinephrine-induced constriction of forearm blood vessels. Whereas, Hurwitz *et al.*[14] have demonstrated that serotonin may either enhance or inhibit the action of norepinephrine in peripheral blood vessels. The fact that serotonin–norepinephrine interaction produces such a wide variety of physiological responses has led to much confusion as to the influence of serotonin on adrenergic functions.

The results of this study indicate that serotonin inhibits norepinephrine uptake in heart tissue. Since amine uptake is apparently the major route of norepinephrine inactivation around the synapse, this antagonism of norepinephrine inactivation could result in a potentiation of norepinephrine response as seen in the study of Scroop and Walsh.[13]

Serotonin also appears to reduce the intraneuronal norepinephrine storage capacity in heart tissue. Fluorescence studies by Lichtensteiger *et al.*[15] also indicate that serotonin is taken up by the catechol-containing neurons, where it is accumulated by the norepinephrine storage granules. Since serotonin is accumulated by the catecholamine storage vesicles, serotonin may have the capacity to diminish adrenergic function by displacing norepinephrine from storage sites or by acting as a "false transmitter".

Serotonin reduces norepinephrine uptake; therefore, an increase in normetanephrine might be anticipated.[16] This study demonstrates, however, that in the presence of reduced uptake, serotonin also inhibits the formation of normetanephrine. Eisenfeld *et al.*[16] have shown that adrenergic receptor blockers inhibit normetanephrine synthesis but do not inhibit solubilized COMT; because serotonin has the same type of activity, it may be postulated that this indole amine is capable of blocking adrenergic receptors. Serotonin has also been shown to inhibit norepinephrine-stimulated adenyl cyclase in erythrocyte ghosts[17] and pineal gland.[18] Since adenyl cyclase and adrenergic receptors are thought to be connatural,[19] the inhibition of cyclase by serotonin supports the concept of serotonin as an adrenergic receptor blocker.

It is apparent that serotonin influences several adrenergic mechanisms and, depending upon the concentration of serotonin and the length of time that it is present in the synaptic region, a variety of effects on physiological functions might be observed. If serotonin is present acutely at low concentrations, an enhanced norepinephrine response would be expected due to norepinephrine uptake inhibition. On the other hand, at high concentrations, serotonin may inhibit the norepinephrine response because of its apparent blocking action on adrenergic receptors. The ability of norepinephrine storage vesicles to accumulate serotonin may lead to a prolonged decrease

in responses to adrenergic nerve stimulation caused by a diminished release of authentic transmitter.

Whether the effect of serotonin on adrenergic functions has any physiological significance is not known; however, it is possible that this proposed dual blockade may, in certain circumstances, regulate catecholamine access to the receptor and thus modulate the action of these amines.

REFERENCES

1. V. J. UUSPÄÄ, *Experientia* **19,** 156 (1963).
2. W. FELDBERG and R. D. MEYERS, *Nature, Lond.* **200,** 1325 (1963).
3. P. GORDON, F. J. HADDY and M. A. LIPTON, *Science, N.Y.* **128,** 531 (1958).
4. S. L. LIGHTMAN and L. L. IVERSEN, *Br. J. Pharmac. Chemother.* **37,** 638, (1969).
5. L. L. IVERSEN, *Br. J. Pharmac. Chemother.* **21,** 523 (1963).
6. I. J. KOPIN, G. HERTTING and E. K. GORDON, *J. Pharmac. exp. Ther.* **138,** 34 (1962).
7. W. D. HORST, I. J. KOPIN and E. R. RAMEY, *Am. J. Physiol.* **215,** 817 (1968).
8. L. L. IVERSEN, J. GLOWINSKI and J. AXELROD, *J. Pharmac. exp. Ther.* **150,** 173 (1965).
9. H. E. MORGAN, M. J. HENDERSON, D. M. REGEN and C. R. PARK *J. biol. Chem.* **236,** 253 (1961).
10. I. J. KOPIN, J. AXELROD and E. GORDON, *J. biol. Chem.* **236,** 2109 (1961).
11. J. R. CROUT, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **248,** 85 (1964).
12. J. AXELROD, W. ALBERS and C. D. CLEMENTE, *J. Neurochem.* **5,** 68 (1959).
13. G. C. SCROOP and J. O. WALSH, *Aust. J. exp. Biol. med. Sci.* **46,** 573 (1968).
14. R. HURWITZ, R. W. CAMPBELL, P. GORDON and F. J. HADDY *J. Pharmac. exp. Ther.* **133,** 57 (1961).
15. W. LICHTENSTEIGER, U. MUTZNES and H. LANGEMANN, *J. Neurochem.* **14,** 489 (1967).
16. A. J. EISENFELD, J. AXELROD and L. KRAKOFF, *J. Pharmac. exp. Ther.* **156,** 107 (1967).
17. H. SHEPPARD and C. R. BURGHARDT, *Molec. Pharmac.* **6,** 425 (1970).
18. B. WEISS and E. COSTA, *J. Pharmac. exp. Ther.* **161,** 310 (1968).
19. G. A. ROBISON, R. W. BUTCHER and E. W. SUTHERLAND, *Ann. N.Y. Acad. Sci.* **139,** 703 (1967).