Effect of desipramine and reserpine on the *in vivo* β-hydroxylation of α-methyl-*m*-tyramine and α-methyldopamine

SIR,—It has been shown that desipramine and related compounds block the amine-uptake mechanism of the adrenergic neuron at the level of the cell membrane ("the membrane pump"). On the other hand reserpine blocks the amine storage mechanism of the amine granules (Hillarp & Malmfors, 1964; Malmfors, 1965; Carlsson & Waldeck, 1965). The enzyme dopamine-β-hydroxylase, which converts dopamine to noradrenaline, appears to be located within the adrenergic neuron (Carlsson & Waldeck, 1963; Fischer, Musacchio, Kopin & Axelrod, 1964) and evidence has been presented that this enzyme is attached to the amine-storing granules (Kirschner, 1962; Potter & Axelrod, 1963).

On the basis of *in vitro* evidence it has been proposed that reserpine inhibits the β-hydroxylation of dopamine (Kirschner, 1962; Weiner & Rutledge, 1966; Rutledge, 1966; Stjärne, 1966). The investigation of this problem *in vivo* is complicated by the fact that any noradrenaline formed in the adrenergic nerve fibres of reserpine-treated animals will probably be deaminated rapidly by intraneuronal monoamine oxidase and the deaminated metabolites promptly escape into the general circulation. We have therefore examined the effect of reserpine on the β-hydroxylation of dopamine analogues containing a methyl group in the α-position which are thus resistant to monoamine oxidase. In this investigation we have also included desipramine. This drug might be expected to inhibit β-hydroxylation of the administered amines by preventing their entry into the adrenergic nerve fibres.

We used tritium-labelled α-methyldopamine and α-methyl-*m*-tyramine. These compounds are good substrates for dopamine β-hydroxylase (Creveling, 1963), and their β-hydroxylation *in vivo* has been demonstrated (Carlsson & Lindqvist, 1962).

In one experimental series, ^{3}H-α-methyldopamine or ^{3}H-α-methyl-*m*-tyramine, synthesized by Hallhagen & Waldeck (to be published) according to the principles of Birkhofer & Hempel (1963), were injected intravenously into mice, some of which had received pretreatment with desipramine (Table 1). The animals were killed 30 min after the injection of the labelled compounds and the ^{3}H-α-methyldopamine and ^{3}H-α-methylnoradrenaline or ^{3}H-α-methyltyramine and ^{3}H-metaraminol in the hearts were isolated by ion-exchange chromatography and measured by liquid scintillation counting. In the hearts of animals given ^{3}H-α-methyltyramine alone the amount of the corresponding β-hydroxylated compound, ^{3}H-metaraminol, was about 20% of the precursor at the time interval

TABLE 1. EFFECT OF DESIPRAMINE ON THE β-HYDROXYLATION OF ^{3}H-α-METHYL-*m*-TYRAMINE AND ^{3}H-α-METHYLDOPAMINE IN THE MOUSE HEART. Desipramine 10 mg/kg was given i.v. 5 min before the i.v. injection of ^{3}H-α-methyltyramine (20 μg/kg) or ^{3}H-α-methyldopamine (40 μg/kg). The animals were killed 30 min after. Controls received labelled compounds only. Each value represents the level in 6 pooled hearts.

Treatment	^{3}H-α-methyltyramine injected		^{3}H-α-methyldopamine injected	
	^{3}H-α-methyltyramine ng/g	^{3}H-metaraminol ng/g	^{3}H-α-methyldopamine ng/g	^{3}H-α-methylnoradrenaline ng/g
Control	12·6	2·4	63·0	25·6
	11·9	1·9	36·7	31·2
Desipramine ..	1·7	0·6	3·2	4·7
	2·0	0·0	1·2	0·7

TABLE 2. EFFECT OF RESERPINE ON THE β-HYDROXYLATION OF ^{3}H-α-METHYL-*m*-TYRAMINE IN THE MOUSE HEART. Reserpine (10 mg/kg) was given i.p. 6 hr before the i.v. injection of ^{3}H-α-methyltyramine (20 μg/kg). The animals were killed 15, 30 and 60 min after. Controls received ^{3}H-α-methyltyramine only. Each value represents the level in 6 pooled hearts.

Time after ^{3}H-α-methyltyramine (min)	Control		Reserpine	
	^{3}H-α-methyltyramine ng/g	^{3}H-metaraminol ng/g	^{3}H-α-methyltyramine ng/g	^{3}H-metaraminol ng/g
15	21·0	2·7	12·4	1·8
	16·8	1·2	12·5	2·1
30	12·3	2·9	6·9	2·0
	—	—	6·2	2·0
60	11·8	4·2	4·2	2·0
	10·0	3·9	4·3	1·8

studied. When ^{3}H-α-methyldopamine was given alone the amount of ^{3}H-α-methylnoradrenaline was about 50% of the precursor. Desipramine given before either labelled compound much diminished the amount of the precursor in the heart as well as that of the product.

In another experimental series, mice pretreated with reserpine were given ^{3}H-α-methyltyramine (Table 2). The animals were killed at different time intervals thereafter. Fifteen min after the injection of ^{3}H-α-methyltyramine its concentration in the hearts of reserpine-treated animals was about 30% lower than in the control hearts. The amount of ^{3}H-metaraminol formed appeared to be about the same in the two groups. From 15 to 60 min after the injection, ^{3}H-α-methyltyramine in the reserpine-treated animals disappeared more rapidly than in the controls. During the same time ^{3}H-metaraminol remained approximately constant in the reserpine-treated animals whereas there was a twofold increase in the controls.

Normally the α-methylated compounds used in the present investigation will be transported into the neuron by the "membrane pump" where the β-hydroxylation probably takes place in or at the amine storage granules. Blockade of the "membrane pump" by desipramine prevented the entrance of ^{3}H-α-methyltyramine and ^{3}H-α-methyldopamine to the β-hydroxylase. Hence little or no ^{3}H-metaraminol or ^{3}H-α-methylnoradrenaline could be detected. In the reserpine-treated animals ^{3}H-α-methyltyramine was still taken up by the "membrane pump" but disappeared more rapidly than in the controls because the granular storage mechanism was blocked. As long as ^{3}H-α-methyltyramine was present in sufficient amounts the β-hydroxylation continued and ^{3}H-metaraminol accumulated. Similar results were obtained with ^{3}H-α-methyldopamine (unpublished data). Inhibition of the β-hydroxylase by a specific inhibitor blocked the formation of metaraminol and α-methylnoradrenaline while there was a simultaneous increase of α-methyltyramine and α-methyldopamine, respectively (unpublished data).

Thus it would appear that β-hydroxylation of α-methyl-*m*-tyramine and α-methyldopamine takes place despite the blockade of the storage mechanism by reserpine. In other words, these α-methylated amines reach the dopamine β-hydroxylase without being incorporated in the reserpine-sensitive storage complex. Evidently more work is needed to clarify the possible relationship between amine storage and β-hydroxylation.

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References

Birkhofer, L. & Hempel, K. (1963). *Chem. Ber.*, **96**, 1373–1381.
Carlsson, A. & Lindqvist, M. (1962). *Acta physiol. scand.*, **54**, 87–94.
Carlsson, A. & Waldeck, B. (1963). *Acta pharmac. tox.*, **20**, 371–374.
Carlsson, A. & Waldeck, B. (1965). *J. Pharm. Pharmac.*, **17**, 243–244.
Creveling, C. R. (1963). In *Pharmacology of Cholinergic and Adrenergic Transmission*. Editors: Koelle, G. B., Douglas, W. W. & Carlsson, A. Oxford, 1965. Pergamon.
Fischer, J. E., Musacchio, J. M., Kopin, I. J. & Axelrod, J. (1964). *Life Sci.*, **3**, 413–419.
Hillarp, N.-Å. & Malmfors, T. (1964). *Ibid.*, **3**, 703–708.
Kirshner, N. (1962). *Science, N.Y.*, **135**, 107–108.
Malmfors, T. (1965). *Acta physiol. scand.*, **64**, Suppl. 248.
Potter, L. T. & Axelrod, J. (1963). *J. Pharmac. exp. Ther.*, **142**, 299–305.
Rutledge, Ch. O. (1966). *Ibid.*, in the press.
Stjärne, L. (1966). *Acta physiol. scand.*, **67**, 441–454.
Weiner, N. & Rutledge, Ch. O. (1966). In *Mechanisms of Release of Biogenic Amines*. Editors: Euler, U. S. von, Rosell, S. & Uvnäs, B. Oxford: Pergamon.

Similar pharmacological properties of ergometrine and methysergide

SIR,—Ergometrine (*N*-[1-(hydroxymethyl)ethyl]-D-lysergamide) and methysergide (*N*-[1-(hydroxymethyl)propyl]-1-methyl-D-lysergamide) are closely related chemically, and we find that they share anti-5-hydroxytryptamine (5-HT) and oxytocic activities.

With the uterus of the rat in di-oestrus, suspended in de Jalon solution at 30°, approximately equal contractions were obtained with 1·0 μg ergometrine and 3·5 μg methysergide. A similar potency ratio was observed using the ileum of the guinea-pig but the doses required to produce contractions were ergometrine 10–50 μg and methysergide 20–200 μg.

In experiments made on three preparations from rats in oestrus, the amounts of the two substances which inhibited 5-HT contractions of the isolated uterus were, methysergide 0·15, 0·2, and 0·3 μg, and ergometrine 0·5, 0·5, and 0·7 μg. The mean potency ratio from these three experiments was methysergide : ergometrine = 2·7 : 1.

These experiments on the uterus of the rat show that ergometrine possesses appreciable anti-5-HT activity, being only 2·7 times less potent than methysergide, while methysergide itself is only 3·5 times less potent than ergometrine in oxytocic activity.

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