The inhibition of catecholamine biosynthesis by apomorphine

Apomorphine stimulates the dopamine receptors in the striatum (Ernst, 1967; Ernst & Smelik, 1966; Andén, Rubenson & others, 1967). More recently, apomorphine was also used in the treatment of parkinsonism (Cotzias, Papavasiliou & others, 1970). Biochemical studies have shown that apomorphine retards the depletion of the central dopamine stores, but not noradrenaline stores, in animals pretreated with tyrosine hydroxylase inhibitors (Andén & others, 1967). Apomorphine reduces the impulse flow of the dopamine neurons, probably by a negative feedback mechanism arising from dopamine receptor stimulation. But no evidence exists on whether apomorphine has a direct effect on tyrosine hydroxylase activity. Apomorphine contains a catechol group and catechols are known to be inhibitors of tyrosine hydroxylase (Nagatsu, Levitt & Udenfriend 1964; Goldstein, Gang & Anagoste, 1967). We have now investigated the effects of apomorphine on tyrosine hydroxylase activity and on dopamine biosynthesis *in vitro* and *in vivo*.

Male Sprague-Dawley rats, 250–300 g, were decapitated and the striata immediately dissected, sliced and incubated at 37° in Krebs-Henseleit medium. The incubation procedure and the determination of [^{14}C]catecholamines was done as previously described (Goldstein, Ohi & Backstrom, 1970). In some experiments the animals were treated with apomorphine 25 mg/kg subcutaneously and 30 min later [^{14}C]L-tyrosine (U) (6·25 μCi/rat; specific activity 450 mCi/mol) was administered intracisternally. Haloperidol (2 mg/kg, i.p.) was given 30 min before the apomorphine. Tyrosine hydroxylase activity was measured according to Nagatsu & others (1964).

The effects of apomorphine on tyrosine hydroxylase activity at different concentrations of the substrate 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$) are in Table 1. Apomorphine 10^{-4}M significantly inhibits tyrosine hydroxylase activity. At lower concentrations of the pteridine the inhibition by apomorphine is more effective. However, at 10^{-6}M, apomorphine does not inhibit significantly tyrosine hydrolyase activity even at low $DMPH_4$ concentrations.

Apomorphine inhibits effectively the biosynthesis of [^{14}C]dopamine from [^{14}C] tyrosine in slices of rat striatum (Table 2) even at 10^{-6} and 10^{-7}M. Thus, striatal slices are more sensitive to the inhibition of [^{14}C]dopamine biosynthesis by apomorphine than tyrosine hydroxylase preparations obtained either from bovine adrenal glands or from the striatum of rats. The addition of haloperidol to the media in which the striatal slices were incubated did not affect the inhibitory activity of apomorphine.

In separate experiments the effects of apomorphine on [^{14}C]catecholamines were investigated after intraventricular injection of [^{14}C]tyrosine. Treatment with apomor-

Table 1. *The effect of apomorphine on tyrosine hydroxylase activity at different concentrations of* $DMPH_4$. The results represent averages from 3 experiments with a standard deviation of $\pm 5\%$. Enzyme preparations at the $(NH_4)_2SO_4$ purification step obtained from bovine adrenal glands and from the striatum of rats were used. The enzyme was preincubated at room temperature (20°) for 5 min with apomorphine and the incubations were according to Nagatsu & others, (1964).

Concentration of apomorphine (M)	Activity % of control Concentration of $DMPH_4$ (μmol) 0·5	0·25	0·1
Control	100·0	100·0	100·0
10^{-6}	100·0	95·5	90·0
10^{-5}	90·0	76·0	70·5
10^{-4}	59·0	51·5	42·5

Table 2. *The effect of apomorphine on* [^{14}C]*dopamine biosynthesis from* [^{14}C]*tyrosine in slices obtained from the stiratum of rats.* In all experiments the slices were incubated for 20 min. Results are the mean ± s.e. from 5 experiments and are expressed as counts/min × 10^{-3} per incubation. Each incubation contained 70 mg of slices.

Concentration of apomorphine (M) in the incubation medium	[^{14}C]Dopamine formed slices	medium	% Inhibition
Control	55·0 ± 1·00	6·8 ± 0·30	——
10^{-7}	44·5 ± 0·70	5·1 ± 0·25	19·8
10^{-6}	22·0 ± 0·50	3·1 ± 0·20	59·4
10^{-5}*	8·5 ± 0·35	2·0 ± 0·20	83·0

* Haloperidol 5 × 10^{-5} M had no effect on the inhibition of [^{14}C]dopamine synthesis by apomorphine.

phine results in approximately 50% decrease of [^{14}C]catecholamine biosynthesis from [^{14}C]tyrosine in the telencephalon and in the brain stem of rats. The *in vivo* inhibitory activity of apomorphine was not affected by pretreatment of the rats with haloperidol.

Apomorphine, like other catechols, inhibits tyrosine hydroxylase activity *in vitro* and *in vivo*. The findings that striatal slices are more sensitive to the inhibition of dopamine biosynthesis by apomorphine than tyrosine hydroxylase preparations *in vitro* suggest that apomorphine accumulates in the striatal dopaminergic neurons or that some other mechanisms are responsible for the effective inhibition in the striatal slices. The experiments with haloperidol, a drug known to block the dopamine receptors, indicate that the inhibitory effectiveness of apomorphine is not due to its stimulation of dopamine receptors. Thus, the inhibitory effectiveness of apomorphine in the striatal slices is most likely due to the accumulation of the drug in the dopaminergic neurons.

Although apomorphine inhibits dopamine formation *in vivo*, it is unlikely that the functional changes produced by the drug are associated with its inhibitory properties. The functional changes are observed after the administration of low doses of apomorphine (Butcher & Andén, 1969), while the inhibition of dopamine biosynthesis *in vivo* requires a higher dose.

The inhibition of catecholamine biosynthesis by apomorphine may limit the usefulness of this drug or of some apomorphine type derivatives in treatment of parkinsonism. The stimulation of the dopamine receptors with a concomitant decrease in catecholamine levels might produce some undesirable effects.

Finally, the present findings suggest an interpretation other than that previously presented for the acceleration of noradrenaline disappearance by apomorphine after tyrosine hydroxylase inhibition (Persson & Waldeck, 1970). The accelerated noradrenaline disappearance produced by apomorphine after tyrosine hydroxylase inhibition is most likely due to the potentiation of tyrosine hydroxylase inhibition and not as previously postulated, to the stimulation of dopamine receptors.

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Sensitivity changes to noradrenaline in the guinea-pig vas deferens induced by amphetamine, cocaine and denervation

Recently de Moraes & Carvalho (1968) and Carvalho, Martins & de Moraes (1970) provided strong evidence that amphetamine is an indirectly-acting sympathomimetic amine that induces presynaptic supersensitivity to noradrenaline. Amphetamine is known to inhibit noradrenaline uptake (Axelrod, Hertting & Potter, 1962; Burgen & Iversen, 1965; Häggendal & Hamberger, 1967). The current theory of the action of cocaine is that the drug produces competitive saturation of the noradrenaline uptake into adrenergic nerves (Furchgott, Kirpekar & others 1963; Draskóczy & Trendelenburg, 1968) by impairing amine uptake by the adrenergic nerves (Langer & Trendelenburg, 1969). On the other hand, the sensitizing action of cocaine cannot be attributed solely to this action

Guinea-pigs 450–600 g were killed by a blow on the back of the neck and decapitated. The vas deferens was suspended in a water-jacketed bath containing 18 ml of modified Krebs-bicarbonate solution (Huković, 1961), maintained at 31° and bubbled with 5% carbon dioxide in oxygen. Dose-response curves for noradrenaline were obtained by the single dose method and constructed from recording of isotonic contractions obtained by means of a frontal writing level on a kymograph. Two control dose-response curves were always determined on each vas deferens before the treatment of the tissue with the sensitizing agent. Tissues were sensitized to noradrenaline with amphetamine or cocaine during 20 min. Repetition of dose-response curves at intervals less than 20 min occasionally resulted in erratic responses. (−)-Noradrenaline bitartrate (+)-amphetamine sulphate and cocaine hydrochloride were dissolved in distilled demineralized water which contains 0·02 mM of ascorbic acid. Noradrenaline, cocaine and amphetamine were expressed as molar concentrations of the bases. In some of the animals the vas deferens was denervated according to Birmingham (1967). Fourteen days after surgical sympathectomy the animals were killed and the vas deferens prepared as described.

The dose-response curves to noradrenaline determined on the preparation before and after the exposure to amphetamine 10^{-4} M, and to cocaine (10^{-5} M) and after surgical denervation and amphetamine (10^{-4} M) are shown in Fig. 1. It is apparent that after the treatment with amphetamine the dose-response curve of the preparation to noradrenaline is shifted to the left by more than 2 log units without increase in the maximum control response; this we have found before (Carvalho, Martins & de Moraes, 1970). After treatment with cocaine or surgical denervation, amphetamine shifts the dose-response curve to noradrenaline to the left only by factors of 20 and 18 respectively, although cocaine and surgical sympathectomy increased the maximum control response (Table 1).