Increase in the turnover of brain dopamine by stimulation of muscarinic receptors outside the dopamine nerve terminals

N.-E. ANDÉN*, H. WACHTEL, *Department of Pharmacology, University of Göteborg, Fack, S-400 33 Göteborg, Sweden*

Centrally acting acetylcholine-like drugs can increase the turnover of brain dopamine. For example, oxotremorine and physostigmine elevate the concentration of homovanillic acid (Laverty & Sharman, 1965; Perez-Cruet, Gessa & others, 1971; Nose & Takemoto, 1974; Andén, 1974a), accelerate the α-methyltyrosine-induced disappearance of dopamine and noradrenaline (Corrodi, Fuxe & others, 1967), enhance the output of dopamine from the caudate nucleus (Bartholini & Stadler, 1975) and increase the accumulation of dopa in the corpus striatum following dopa decarboxylase inhibition (Javoy, Agid & Glowinski, 1975). These effects are probably due to stimulation of muscarinic receptors in the brain since they are prevented by centrally active (e.g., atropine), but not by peripherally active (e.g., methylatropine), anti-muscarinic drugs. The muscarinic agonists may increase the turnover of dopamine by an action on the dopamine nerve terminals but they could produce this effect also by accelerating the nerve impulse flow in the dopamine neurons. One way to differentiate between these alternatives is to interrupt the nerve impulse flow in the dopamine neurons. The γ-hydroxybutyrate precursor, γ-butyrolactone completely inhibits the normal firing of the dopamine cells in the mesencephalon (Roth, Walters & Aghajanian, 1973) as well as the increased firing following neuroleptic administration (Walters & Roth, 1976). In the present work, the tyrosine hydroxylase activity was determined *in vivo* in the dopamine-rich corpus striatum and in a noradrenaline-predominant area of the forebrain after treatment with oxotremorine or physostigmine in combination with γ-butyrolactone or apomorphine + γ-butyrolactone. It should be noted that the dopa formation from tyrosine in the dopamine neurons can be enhanced both in the absence of nerve impulses as well as at an increased depolarization (Andén, Magnusson & Stock, 1974).

Male Sprague-Dawley rats, 155–230 g, kept at 31° and with a rectal temperature of approximately 37° were used. Methylatropine (1 mg kg^{-1}, i.p.) was given in most cases to prevent peripheral muscarinic effects. The accumulation of dopa following complete inhibition of dopa decarboxylase by 3-hydroxybenzylhydrazine (NSD 1015; 100 mg kg^{-1}, i.p., 30 min) was used as an index of tyrosine hydroxylase activity (Carlsson, Davis & others, 1972). The rats were decapitated under light chloroform anaesthesia and the brains quickly removed and dissected on an ice-cooled glass plate under a microscope. The dopamine-rich corpus striatum and the noradrenaline-predominant remainder of the forebrain (neocortex + hippocampus + thalamus + hypothalamus) from each rat were collected, the rest of the limbic system, the cerebellum and the lower brain stem being discarded. After homogenization, cation exchange chromatography and oxidation, dopa was determined spectrofluorimetrically (Kehr, Carlsson & Lindqvist, 1972). The following drugs were used: oxotremorine (Aldrich, Beerse), physostigmine salicylate (Sigma, St. Louis), *N*-methylatropine hydroiodide (synthesized in this department), atropine sulphate (Sigma, St Louis), apomorphine HCl (Sandoz, Basle), γ-butyrolactone (Merck, Darmstadt), 3-hydroxybenzylhydrazine HCl (NSD 1015; synthesized in this department). All doses refer to the forms indicated.

The dopa concentrations after dopa decarboxylase inhibition in the dopamine-predominant corpus striatum are presented in Table 1. Methylatropine did not affect the dopa accumulation. Oxotremorine and physostigmine increased the dopa accumulation in the corpus striatum. This effect was blocked by atropine indicating stimulation of central muscarinic receptors. Interruption of the nerve impulse flow by γ-butyrolactone

Table 1. *Effects of oxotremorine (O, 2·5 mg kg^{-1}, i.p., 45 min), physostigmine (P, 1·5 mg kg^{-1}, i.p., 45 min), methylatropine (M, 1 mg kg^{-1}, i.p., 60 min), atropine (At, 40 mg kg^{-1}, i.p., 60 min), apomorphine (Ap, 2 mg kg^{-1}, i.p., 40 min) and γ-butryolactone (G, 750 mg kg^{-1} i.p., 30 min) on the dopa accumulation (μg g^{-1}) in the corpus striatum of rats induced by 3-hydroxybenzylhydrazine (N, NSD 1015, 100 mg kg^{-1}, i.p., 30 min).*

Treatment	Dopa accumulation*	Difference†
1 M + N	1·07 (11)	
2 M + O + N	1·74 (8)	2–1: $P < 0.001$
3 M + P + N	1·80 (8)	3–1: $P < 0.001$
4 N	0·99 (12)	4–1: $P > 0.05$
5 At + O + N	0·90 (7)	5–2: $P < 0.001$
		5–4: $P > 0.05$
6 At + P + N	0·89 (6)	6–3: $P < 0.001$
		6–4: $P > 0.05$
7 M + G + N	3·07 (6)	7–1: $P < 0.001$
8 M + O + G + N	2·81 (7)	8–7: $P > 0.05$,
		8–2: $P < 0.001$
9 M + P + G + N	3·07 (7)	9–7: $P > 0.05$
		9–3: $P < 0.001$
10 M + Ap + N	0·64 (6)	10–1: $P < 0.05$
11 M + O + Ap + N	0·78 (7)	11–10: $P > 0.05$
		11–2: $P < 0.001$
12 M + P + Ap + N	0·74 (7)	12–10: $P > 0.05$
		12–3: $P < 0.001$
13 M + Ap + G + N	1·04 (6)	13–7: $P < 0.001$
		13–10: $P < 0.05$
14 M + O + Ap + G + N	0·86 (6)	14–13: $P > 0.05$
		14–2: $P < 0.001$
15 M + P + Ap + G + N	0·92 (7)	15–13: $P > 0.05$
		15–3: $P < 0.001$

* Mean with number of experiments in parentheses.
† Statistical significances by one-way analysis of variance followed by *t*-test ($F = 42.238$, d.f. within groups = 96, variance within groups = 0·121774).

* Correspondence.

markedly increased the dopa concentration and the two acetylcholine-like agents did not further enhance the γ-butyrolactone-induced increase. The dopa formation was slightly reduced by apomorphine and this effect was not significantly influenced when oxotremorine and physostigmine were given in combination with apomorphine. Apomorphine given together with γ-butyrolactone lowered the dopa accumulation to approximately normal concentrations. Oxotremorine and physostigmine did not stimulate dopa synthesis following apomorphine + γ-butyrolactone. In fact, there was a significant reduction by both oxotremorine ($P < 0.025$) and physostigmine ($P < 0.05$) when Student's *t*-test was used for comparisons with group 13 in Table 1.

The results from the noradrenaline-predominant parts of the forebrain are presented in Table 2. The effects of oxotremorine and physostigmine were similar to those described above for the dopamine-rich corpus striatum. The main exceptions were that the stimulations were about the same following oxotremorine, physostigmine or γ-butyrolactone and that apomorphine was almost ineffective by itself and in combination with oxotremorine or physostigmine.

The inhibition by apomorphine of the γ-butyrolactone-induced increase in dopa accumulation in the corpus striatum is probably due to stimulation of presynaptic dopamine receptors in a similar way to that described following axotomy (Kehr, Carlsson & others, 1972; Roth & others, 1973; Walters & Roth, 1976). Since the enhancement of the dopa formation by oxotremorine and physostigmine was not observed following apomorphine + γ-butyrolactone, it appears that the stimulation by both acetylcholine-like agents is dependent on nerve impulses. Therefore, oxotremorine and physostigmine might act by increasing the nerve impulse flow in the dopamine nerves. Alternatively, these agents might also facilitate the stimulatory effect on dopamine synthesis per impulse. However, activation of muscarinic receptors by acetylcholine in electrically stimulated slices from the rat corpus striatum does not increase the release of dopamine induced by nerve impulses (Westfall, 1974). These findings indicate that the muscarinic receptors are probably not localized on the terminals of the dopamine neurons. They might occur in the substantia nigra (Smelik & Ernst, 1966; Aghajanian & Bunney, 1973; Javoy, Agid & others, 1974) or on non-dopamine-containing structures in the corpus striatum or elsewhere. The stimulation of these muscarinic receptors outside the dopamine nerve terminals might increase the nerve impulse flow in the dopamine neurons.

Table 2. *Effects of oxotremorine (O, 2·5 mg kg^{-1}, i.p. 45 min), physostigmine (P, 1·5 mg kg^{-1}, i.p., 45 min), methylatropine (M, 1 mg kg^{-1}, i.p., 60 min), atropine (At, 40 mg kg^{-1}, i.p., 60 min), apomorphine (Ap, 2 mg kg^{-1}, i.p., 40 min) and γ-butyrolactone (G, 750 mg kg^{-1}, i.p., 35 min) on the dopa accumulation (μg g^{-1}) in the rest of the forebrain (the forebrain without the corpus striatum and the limbic system) of rats induced by 3-hydroxybenzylhydrazine (N, NSD 1015, 100 mg kg^{-1}, i.p., 30 min).*

Treatment	Dopa accumulation*	Difference†
1 M + N	0·102 (11)	
2 M + O + N	0·190 (8)	2–1: $P < 0.001$
3 M + P + N	0·169 (8)	3–1: $P < 0.001$
4 N	0·093 (12)	4–1: $P > 0.05$
5 At + O + N	0·093 (7)	5–2: $P < 0.001$ 5–4 $P > 0.05$
6 At + P + N	0·113 (6)	6–3: $P < 0.001$ 6–4: $P > 0.05$
7 M + G + N	0·169 (6)	7–1: $P < 0.001$
8 M + O + G + N	0·196 (7)	8–7: $P > 0.05$ 8–2: $P > 0.05$
9 M + P + G + N	0·165 (7)	9–7: $P > 0.05$ 9–3: $P > 0.05$
10 M + Ap + N	0·089 (6)	10–1: $P > 0.05$
11 M + O + Ap + N	0·169 (7)	11–10: $P < 0.001$ 11–2: $P > 0.05$
12 M + P + Ap + N	0·133 (7)	12–10: $P < 0.01$ 12–3: $P < 0.05$
13 M + Ap + G + N	0·121 (6)	13–7: $P < 0.01$ 13–10: $P > 0.05$
14 M + O + Ap + G + N	0·152 (6)	14–13: $P > 0.05$ 14–2: $P < 0.05$
15 M + P + Ap + G + N	0·120 (7)	15–13: $P > 0.05$ 15–3: $P < 0.01$

* Mean with number of experiments in parentheses.
† Statistical significances by one-way analysis of variance followed by *t*-test ($F = 11·607$, d.f. within groups = 96, variance within groups = 0·0860220).

The small reduction by oxotremorine and physostigmine of the dopa accumulation following apomorphine + γ-butyrolactone might indicate that there are inhibitory rather than excitatory muscarinic receptors on the dopamine nerve terminals similar to those described on noradrenaline nerve terminals (Muscholl, 1973).

The absence of a potentiation of the γ-butyrolactone-induced increase in the dopa formation in the corpus striatum by oxotremorine or physostigmine can be explained; (i) if the two acetylcholine-like agents are not able to antagonize the γ-butyrolactone-induced cessation of the nerve impulse flow or (ii) if the treatment with γ-butyrolactone by itself had maximally stimulated the tyrosine hydroxylase activity. The failure of oxotremorine and physostigmine to influence the dopa accumulation following apomorphine is in agreement with other findings (van Zwieten-Boot & Petri-Bot, 1976) and might be due to a complete inhibition of the nerve impulse flow in the dopamine neurons by apomorphine (Aghajanian & Bunney, 1973).

The similar stimulation of the dopa accumulation in the noradrenaline and in the dopamine neurons by both oxotremorine and physostigmine indicates that the nerve impulse flow is increased also in the noradrenaline neurons. In fact, oxotremorine and physostigmine have been shown to accelerate the turnover of noradrenaline in the rat brain (Corrodi & others, 1967; Kažić, 1973). γ-Butyrolactone also increases the synthesis (Table 2) and the utilization (Andén, 1974b) of brain noradrenaline and this effect might explain the lack of an additional stimulation by oxotremorine or physostigmine.

In conclusion, oxotremorine and physostigmine in-

crease the dopa accumulation following dopa decarboxylase inhibition in both dopamine and noradrenaline neurons indicating that these drugs increase the synthesis of both amines. The effect on the dopamine neurons is probably evoked by stimulation of muscarinic receptors outside the dopamine nerve terminals leading to enhancement of the nerve impulse flow.

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Modification of electroshock fighting by drugs known to interact with dopaminergic and noradrenergic neurons in normal and brain lesioned rats

M. ANAND*, G. P. GUPTA†, K. P. BHARGAVA†, *Industrial Toxicology Research Centre, Lucknow, and †Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow, India*

The stimulation of certain brain areas—septum, amygdala and lateral hypothalamus, results in marked changes in the electroshock induced fighting behaviour in animals (Anand & Brobeck, 1951; Brady & Nauta, 1953; Woods, 1956; Morgane & Kosman, 1957; Morrison, Barrnett & Mayer, 1958; Ahmad & Harvey, 1968). Changes in the concentration or metabolism of cerebral catecholamines modify the behavioural pattern (Strom-Olsen & Weil Malherbe, 1958; Schild Krant & Kety, 1967; Rubin, 1968). Both adrenaline and dopamine are believed to be involved in the production of aggressiveness (Randrup & Munkvad, 1969; Eichelman, Thoa & Andén, 1972). The present report is concerned with the effect of certain drugs which interact with

* Correspondence.