Effect of several dopaminergic drugs and trihexyphenidyl on cholinergic parameters in the rat striatum

Although little is known about the function of acetylcholine in extrapyramidal structures in the brain, a cholinergic mechanism may be involved in some symptoms of parkinsonism as evidenced by the efficacy of antimuscarinic drugs in this disease. In parkinsonism, the striatal dopamine deficiency probably upsets the suggested cholinergic-dopaminergic balance in this area resulting in a preponderance of cholinergic tone and producing the symptoms characteristic of this disease (Koelle, 1972). This hypothesis is based upon indirect, mainly pharmacological evidence and only a few attempts have been made to study cholinergic parameters during treatment with anti-parkinson agents (Sethy & Van Woert, 1973a, b; Kim, Katoaka & others, 1972).

This study was therefore undertaken to determine the effect of several direct and indirect acting dopamine-like drugs, as well as the antimuscarinic agent trihexyphenidyl, on some cholinergic parameters in the striatum.

Female Sprague-Dawley rats, 210 ± 10 g, were decapitated and the head was immediately immersed in liquid nitrogen for 6–7 s. Each brain was placed in n-pentane at −3° (Campbell & Jenden, 1970) for isolation of the left corpus striatum under the dissecting microscope. Less than 150 s were required for this step. Isolated single striata consisted of the putamen nucleus, caudate nucleus and globus pallidus nucleus and had a mean weight of 49·0 ± 0·7 mg (mean ± s.e.). After isolation, the striata were frozen immediately in liquid nitrogen, weighed in the frozen state and pulverized (Ladinsky, Consolo & Sanvito, 1972a). The radiochemical assay of Saelens, Allen & Simke (1970), with slight modifications (Ladinsky, Consolo & Bareggi, 1972b), was then followed for the estimation of acetylcholine and choline. Acetylcholinesterase activity was determined by the radiochemical method of McCaman, Tomey & McCaman (1968). The drugs used were: L-dopa (gift from Lepetit, S.p.A.) dissolved in 0·01N HCl; 1-(2″-pyrimidyl)-4-piperonyl-piperazine (piribedil, ET 495, Trivastal; gift from the Servier Laboratories, Paris), dissolved in 0·5N HCl; amantadine HCl (Symmetrel; gift from DuPont), dissolved in distilled water; desipramine (gift from Geigy, Italia) dissolved in distilled water; trihexyphenidyl (Artane; gift from Lederle Laboratories) suspended in 0·5% carboxymethylcellulose; (+)-amphetamine sulphate (gift from Recordati, Milan) dissolved in distilled water. The drugs were administered intraperitoneally in a volume equivalent to 1 ml kg^{-1} weight at the doses and times specified in Table 1.

Table 1 shows the time courses and dose-response effects of these drugs on striatal acetylcholine and choline levels. L-Dopa (100 and 200 mg kg^{-1}), desipramine (10 mg kg^{-1}), amantadine (25 and 50 mg kg^{-1}), apomorphine (2 mg kg^{-1}), piribedil (60 mg kg^{-1}) and (+)-amphetamine (10 mg kg^{-1}) increased striatal acetylcholine levels. The activity of all of these drugs lasted about 120 min with the exception of piribedil which lasted up to about 480 min. Choline levels were unaffected by L-dopa, amphetamine and desipramine while they were increased by about 30% after treatment with amantadine, apomorphine and piribedil. Trihexyphenidyl (10 mg kg^{-1}), on the other hand, markedly decreased striatal acetylcholine levels without affecting choline. The activity lasted for at least 360 min.

The drugs did not inhibit acetylcholinesterase activity of the 500 × *g* fraction of striatal homogenates at an *in vitro* concentration of 10 μg per 2 mg wet wt per ml.

This work has shown that L-dopa, amantadine and amphetamine, drugs which increase the available dopamine pool by different mechanisms (Heikkila & Cohen, 1972; Creese & Iversen, 1973), and amantadine, apomorphine and piribedil, which stimulate dopamine receptors directly (Corrodi, Fuxe & Ungerstedt, 1971; Corrodi, Farnebo & others, 1972; Garattini, Fanelli & others, 1974; Maj, Sowinska & Baran,

Table 1. *Time courses of the dopaminergic drugs and trihexyphenidyl on acetylcholine levels in the rat striatum.*

Drug	Dose (mg kg^{-1}, i.p.)	Time (min)	Acetylcholine (μg g^{-1} wet wt)	Choline (μg g^{-1} wet wt)
0·01N HCl	—	—	3·46 ± 0·16 (9)	10·66 ± 0·52 (12)
L-Dopa	100	15	*4·86 ± 0·24 (5)	11·16 ± 0·19 (5)
L-Dopa	100	30	*6·06 ± 0·76 (9)	9·32 ± 0·42 (10)
L-Dopa	100	60	*5·95 ± 0·51 (10)	10·18 ± 0·43 (10)
L-Dopa	100	120	*4·08 ± 0·20 (9)	10·58 ± 0·64 (7)
L-Dopa	100	240	3·31 ± 0·11 (4)	9·82 ± 0·71 (6)
0·01N HCl	—	—	3·34 ± 0·08 (12)	9·56 ± 0·32 (15)
L-Dopa	200	15	*4·96 ± 0·20 (5)	—
L-Dopa	200	30	*4·88 ± 0·13 (13)	9·03 ± 0·39 (15)
L-Dopa	200	60	*4·25 ± 0·29 (13)	8·98 ± 0·49 (13)
L-Dopa	200	120	*4·42 ± 0·46 (6)	10·50 ± 1·11 (8)
L-Dopa	200	240	3·44 ± 0·20 (4)	—
Saline	—	—	3·41 ± 0·11 (6)	9·79 ± 0·32 (6)
(+)-Amphetamine	10	30	*5·14 ± 0·51 (4)	9·47 ± 0·22 (4)
(+)-Amphetamine	10	60	*5·39 ± 0·28 (8)	10·41 ± 0·71 (9)
(+)-Amphetamine	10	120	*4·88 ± 0·21 (5)	9·94 ± 0·23 (4)
Saline	—	—	3·54 ± 0·08 (4)	10·62 ± 0·28 (25)
Amantadine	25	20	*4·20 ± 0·24 (7)	11·52 ± 0·54 (11)
Amantadine	25	60	*4·12 ± 0·16 (7)	*12·79 ± 0·33 (22)
Amantadine	25	120	3·83 ± 0·38 (8)	*12·69 ± 0·52 (17)
Saline	—	—	3·36 ± 0·05 (10)	10·44 ± 0·24 (17)
Amantadine	50	30	*3·98 ± 0·23 (6)	11·08 ± 0·88 (5)
Amantadine	50	60	*4·03 ± 0·20 (5)	11·96 ± 0·62 (12)
Amantadine	50	120	3·57 ± 0·26 (5)	*13·35 ± 0·34 (14)
Saline	—	—	3·27 ± 0·27 (8)	9·86 ± 0·55 (8)
Apomorphine	2	15	*4·99 ± 0·51 (5)	11·04 ± 0·60 (5)
Apomorphine	2	30	*6·55 ± 0·55 (8)	*11·69 ± 0·39 (8)
Apomorphine	2	60	*8·03 ± 1·09 (5)	*13·56 ± 0·70 (5)
Apomorphine	2	120	*4·57 ± 0·23 (5)	10·63 ± 0·51 (5)
0·5N HCl	—	—	3·45 ± 0·12 (10)	10·87 ± 0·49 (14)
Piribedil	60	15	*8·07 ± 0·74 (10)	10·37 ± 0·61 (10)
Piribedil	60	30	*8·09 ± 0·55 (10)	*12·29 ± 0·45 (11)
Piribedil	60	60	*8·60 ± 0·53 (10)	*13·95 ± 0·78 (12)
Piribedil	60	120	*8·72 ± 0·75 (8)	*14·15 ± 1·26 (9)
Piribedil	60	240	*7·19 ± 0·53 (10)	*12·65 ± 0·77 (12)
Piribedil	60	360	*4·74 ± 0·13 (5)	10·52 ± 0·36 (5)
Piribedil	60	480	*4·38 ± 0·18 (5)	11·10 ± 0·23 (6)
Piribedil	60	960	3·51 ± 0·14 (6)	—
Saline	—	—	3·40 ± 0·05 (28)	10·26 ± 0·25 (38)
Desipramine	10	30	*3·79 ± 0·01 (20)	9·95 ± 0·24 (21)
Desipramine	10	60	*4·13 ± 0·13 (18)	11·07 ± 0·45 (20)
Desipramine	10	120	*3·74 ± 0·02 (5)	10·88 ± 0·64 (11)
Desipramine	10	240	3·45 ± 0·48 (10)	10·24 ± 0·15 (6)
0·5% CMC	—	—	3·38 ± 0·10 (13)	10·48 ± 0·44 (15)
Trihexyphenidyl	10	30	*1·99 ± 0·19 (9)	10·27 ± 0·42 (17)
Trihexyphenidyl	10	60	*1·67 ± 0·10 (11)	11·59 ± 0·71 (17)
Trihexyphenidyl	10	120	*1·93 ± 0·13 (6)	9·58 ± 0·77 (5)
Trihexyphenidyl	10	240	*2·11 ± 0·06 (7)	9·07 ± 0·64 (6)
Trihexyphenidyl	10	360	*2·02 ± 0·18 (5)	9·69 ± 0·26 (5)
Trihexyphenidyl	10	480	3·35 ± 0·11 (5)	10·21 ± 0·24 (6)

* $P < 0·01$.
Values are the mean and s.e. Figures in parentheses represent the number of animals used.

1972), increased acetylcholine levels in the striatum. Desipramine, a drug found to improve the clinical picture of Parkinson's disease (Muscettola, Giovannucci & others, 1972), has been suggested to block uptake of dopamine (Pscheidt & Himwich, 1968) but required high doses and particular conditions. However, desipramine does have other central effects, such as blocking uptake of catecholamines and possessing both central antagonism and the ability to potentiate muscarinic activity (Bevan, Bradshaw & others, 1973).

Our *in vitro* study with doses of these agents, probably much higher than would be found in the brain *in vivo*, excluded the possibility that these drugs increased acetylcholine levels by blocking striatal acetylcholinesterase activity. Thus, either the synthesis of acetylcholine was increased or the drugs acted indirectly by blocking the release of this quaternary amine. The latter hypothesis appears more likely and has been previously proposed, at least for L-dopa, by Sethy & Van Woert (1973a). Furthermore, Stadler, Lloyd & others (1973) demonstrated that apomorphine reversed the chlorpromazine-induced release of acetylcholine from the cat superfused striatum. These data further support the theory (Kim, 1973) that the dopamine pathway modulates the action of cholinergic neurons by inhibitory input. Our findings that trihexyphenidyl decreased striatal acetylcholine levels fit well into this framework since this antimuscarinic agent may block inhibitory cholinergic sites thus freeing the cholinergic fibres and producing increased release of acetylcholine.

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