Dopaminergic potency of apomorphine homologues in mice with unilateral lesions of the caudate nucleus

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The *N*-propyl homologue of apomorphine appeared to be more potent than apomorphine in improving symptoms of parkinsonism in man (Cotzias, Papavasiliou & others, 1976). Likewise, the *N*-propyl homologue was more potent than apomorphine in producing postural asymmetries in mice (Ginos, Cotzias & others, 1975; Pearl, Schumann & Wood, 1976) and rats (Neumeyer, Dafeldecker & others, 1977) with unilateral lesions of the nigrostriatal system. The dopaminergic potency of several apomorphine homologues in mice with unilateral lesions of the caudate nucleus has now been assessed and the results compared with those of others who used different indicants of dopaminergic activity.

Male Swiss-Webster mice (Taconic Farms, N.Y.), 20–23 g at the time of lesioning and 36–45 g at testing, had unilateral lesions of the caudate nucleus made by suction according to Lotti (1971).

Drugs were dissolved in water, and oral, intraperitoneal and subcutaneous medications were 0·01 ml g^{-1}. Observations, 2 min maximum duration, took place immediately before and 15, 30, 60, 120 and 240 min after medication. None of the mice exhibited postural asymmetry toward the lesioned side before medication. Mice were scored positive if they showed postural asymmetry toward the lesioned side during any of the observation period after medication. The ED50 values in mg kg^{-1} of free base were calculated with a computer according to the quantal method described by Finney (1964). At least three doses of each drug were used to estimate ED50 values.

Apomorphine HCl was kindly donated by Merck Sharp and Dohme Research Laboratories. The three apomorphine homologues came from Sterling-Winthrop Research Institute: *N*-ethylnorapomorphine HCl, *N*-n-propylnorapomorphine HCl, *N*-n-butylnorapomorphine HCl. Because all of the compounds were derived from morphine, they belong to the (*R*) - (−) series (Atkinson, Bullock & others, 1975).

Table 1. *Potency of apomorphine homologues in mice with unilateral lesions of caudate nucleus*[a].

N-substituent	ED50 (95% limits) in mg kg^{-1} base	Slope	Potency ratio
	Oral		
n-Propyl	0·63 (0·19–2·08)	2·5[b]	44
Ethyl	9·88 (8·41–11·5)	9·6	3
Methyl	27·8 (21·3–36·3)	6·2	1
n-Butyl	Inactive 50	—	—
	Intraperitoneal		
n-Propyl	0·034 (0·028–0·040)	10·7[c]	27
Ethyl	0·088 (0·023–0·12)	3·1	10
Methyl	0·92 (0·82–1·09)	6·0	1
n-Butyl	Inactive 6·4	—	—
	Subcutaneous		
n-Propyl	0·014 (0·010–0·019)	5·7	16
Methyl	0·23 (0·22–0·26)	12·1	1
n-Butyl	Inactive 100	—	

[a]Mice were observed from 15 to 240 min after medication for postural asymmetries directed toward the side of the lesion. The data for the propyl and methyl compounds were reported previously (Pearl & others, 1976).

[b]The slope of the propyl compound was significantly different from those of the ethyl and methyl compounds ($P < 0·05$).

[c]The slope of the propyl compound was significantly different from that of the ethyl compound ($P < 0·05$).

Table 1 shows the ED50 values of the drugs and their potency ratios relative to apomorphine. Potency increased as the *N*-carbon chain length increased, reached maximum at propyl and vanished at butyl: propyl > ethyl > methyl (apomorphine) > butyl.

Table 2 shows the effects of the three active compounds at different intervals after medication. The peak effect appeared to be 15 min after subcutaneous and intraperitoneal injection of the compounds and from 15 to 30 min after oral medication. At the time of peak effect the ED50 values of the compounds were virtually identical to the ED50 values shown in Table 1. At equiactive doses no appreciable difference in duration of activity of the compounds was evident.

Results for the dopaminergic potency of the apomorphine homologues agree with some of but not all of the previous results. The butyl homologue was consistently inactive in producing postural asymmetries in lesioned mice (present study) and in producing emesis in dogs (Atkinson & others, 1975).

The ethyl homologue was consistently more potent than apomorphine in producing postural asymmetries in lesioned mice (present study), gnawing in mice (Koch, Cannon & Burkman, 1968) and emesis in dogs (Atkinson & others, 1975; Koch & others, 1968). The ethyl homologue was more potent than the propyl homologue in producing emesis in dogs (Atkinson & others, 1975; Koch & others, 1968) and gnawing in mice (Koch & others, 1968) but not in producing asymmetries in mice with unilateral lesions of the caudate nucleus (present study).

The propyl homologue was more potent than apomorphine in producing postural asymmetries in mice with unilateral caudate lesions made by suction (Ginos & others, 1975; Pearl & others, 1976) and in rats with substantia nigra lesions made by electrolytic

Table 2. Effects of apomorphine homologues in caudate-lesioned mice at different time intervals after medication.

N-substituent	Dose mg kg^{-1} base	No. mice tested	% of mice responding at time in min after dose: 15	30	60	120	240
		Oral					
n-Propyl	0·2	6	0	0	0	0	0
	0·4	12	43	43	25	0	0
	0·8	8	50	50	37	37	37
	1·0	4	75	75	50	25	0
	1·6	12	75	75	75	33	33
Ethyl	5	4	0	0	0	0	0
	10	4	50	50	50	0	0
	12	5	80	80	80	20	0
	20	4	100	100	100	10	0
Methyl	16	12	8	8	8	8	0
	32	10	60	60	40	30	20
	64	12	93	100	93	75	17
		Intraperitoneal					
n-Propyl	0·025	8	12	12	0	0	0
	0·035	10	50	50	30	0	0
	0·05	8	100	87	25	0	0
Ethyl	0·05	4	25	25	0	0	0
	0·10	4	50	25	0	0	0
	0·17	6	83	83	17	0	0
Methyl	0·4	8	0	0	0	0	0
	0·8	8	25	12	12	0	0
	1·1	10	50	30	0	0	0
	1·6	8	100	75	37	0	0
		Subcutaneous					
n-Propyl	0·01	10	20	10	0	0	0
	0·02	10	80	80	20	0	0
	0·04	5	100	100	20	0	0
Methyl	0·125	5	0	0	0	0	0
	0·25	12	67	67	0	0	0
	0·5	5	100	100	0	0	0

coagulation or by 6-hydroxydopamine (Costall, Naylor & Neumeyer, 1975; Mendez, Cotzias & others, 1975; Neumeyer & others, 1977), and stereotyped biting in rats (Costall & others, 1975; Schoenfeld, Neumeyer & others, 1975). Whilst the propyl homologue, orally, was more potent than apomorphine in producing postural asymmetries in mice with caudate lesions induced by 6-hydroxydopamine, this was not so when the aporphines were given subcutaneously (Pearl & others, 1976), whilst the propyl analogue was more potent than apomorphine in producing stereotyped biting in monkeys (Atkinson & others, 1975) and in rats (Costall & others, 1975) this was not evident for stereotyped sniffing in rats (Costall & others, 1975) or gnawing in mice (Koch & others, 1968), and whilst in one study the propyl homologue was a more potent emetic than apomorphine in dogs (Atkinson & others, 1975), this was not so in another study (Koch & others, 1968). Further, the propyl homologue, injected into various regions of rat brain, was not substantially more potent than apomorphine in producing biting (Costall, & others, 1975) and, *in vitro*, not more potent than apomorphine in stimulating striatal adenylate cyclase (Miller, Kelly & Neumeyer, 1976).

Concerning the interpretation of differences in potency between apomorphine and its homologues, several considerations may be applicable. For example, the lipophilic nature of the propyl homologue may enhance entry into brain (Burkman, Notari & Van Tyle, 1974). That the propyl homologue was more potent than apomorphine in some of but not all of the tests of dopaminergic activity may point to the existence of different types of dopamine receptors in the extra-pyramidal as opposed to other areas of the brain (Cools & van Rossum, 1976; Neumeyer & others, 1977).

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Is the low incidence of extrapyramidal side-effects of antipsychotics associated with antimuscarinic properties?

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Recent developments in binding assays have made it possible to test if one or several types of receptors are involved in the mechanism of action of psychotropic drugs. Certain neuroleptic drugs besides their well known dopamine blocking activity have also been reported to have antiacetylcholine properties (Miller & Hiley, 1974; Snyder, Greenberg & Yamamura, 1974; Sayers & Burki, 1976). Since the extrapyramidal side-effects of neuroleptics are successfully controlled by antiacetylcholine drugs, it has been suggested that the low incidence of parkinsonian side-effects seen with some antipsychotic drugs could be due to their antimuscarinic properties (Miller & Hiley, 1974; Snyder & others, 1974). Surprisingly, pimozide has also been classified as a member of the group of drugs possessing considerable affinity for muscarinic binding sites (Yamamura, Manian & Snyder, 1976) although the drug has no apparent antiacetylcholine properties (Janssen, Niemegeers & others, 1968).

We now report results dealing with the *in vitro* and *in vivo* binding of various drugs on brain cholinergic receptors using [^{3}H]dexetimide, a potent antimuscarinic agent (Janssen, Niemegeers & others, 1971) as ligand. The potency of neuroleptic drugs was assessed by their affinity in the [^{3}H]haloperidol binding assay.

Male Wistar rats (approximately 250 g) were decapitated and striata removed and homogenized in 10 volumes of distilled water. Binding assay conditions for the brain cholinergic receptor were as follows; 100 μl of a total homogenate diluted 1 : 100 (w/v), [^{3}H]-dexetimide (spec. act. 17·5 Ci mmol^{-1}) 2×10^{-9} M, phosphate buffer (Na/K) 0·05 M pH 7·4 in a total volume of 2·2 ml. After incubation for 20 min at 37°, 3 ml of cold buffer was added, the samples were rapidly filtered under suction through Whatman GF/B glass fibre filters and rinsed twice with 5 ml of cold buffer. The radioactivity on the filter was determined by liquid scintillation counting. Stereospecifically displaceable binding was calculated as the difference between the labelling in the presence of 2×10^{-7} M of (−)-benzetimide (inactive enantiomer) and of (+)-benzetimide (dexetimide) (Soudijn, Van Wijngaarden & Ariëns, 1973). [^{3}H]Haloperidol binding was measured as described by Leysen, Tollenaere & others (1977a). *In vivo* binding or displacement experiments were carried out following a procedure described for neuroleptic drugs (Laduron & Leysen, 1977).

Table 1 shows that [^{3}H]dexetimide binding in rat brain has the characteristics of a muscarinic receptor;

Table 1. *Drug affinity for muscarinic and neuroleptic binding sites in rat striatum.*

Drug	Receptor binding IC50 (M)		Ratio*
	[^{3}H]Dexetimide	[^{3}H]Haloperidol	
Dexetimide	$2·8 \pm 0·1 \times 10^{-9}$	$>10^{-4}$	—
Scoploamine	$4·2 \pm 0·3 \times 10^{-9}$	—	—
Atropine	$6·8 \pm 0·7 \times 10^{-9}$	$>10^{-4}$	—
Isopropamide	$7·5 \pm 0·6 \times 10^{-9}$	—	—
Benztropine	$1·4 \pm 0·2 \times 10^{-8}$	—	—
Trihexedyl	$2·1 \pm 0·2 \times 10^{-8}$	—	—
Clozapine	$1·2 \pm 0·1 \times 10^{-7}$	$4·0 \pm 1·6 \times 10^{-7}$	0·3
Thioridazine	$3·2 \pm 0·1 \times 10^{-7}$	$4·0 \pm 1·6 \times 10^{-8}$	8
Perlapine	$3·6 \pm 0·6 \times 10^{-7}$	$3·2 \pm 1·6 \times 10^{-7}$	1·1
Chlorpromazine	$6·6 \pm 0·6 \times 10^{-7}$	$1·3 \pm 1·3 \times 10^{-7}$	5·1
Promazine	$7·7 \pm 0·6 \times 10^{-7}$	$2·5 \pm 1·6 \times 10^{-7}$	3·1
Pimozide	$4·5 \pm 1·2 \times 10^{-6}$	$3·2 \pm 1·3 \times 10^{-9}$	1406
Pipamperone	$1·0 \pm 0·1 \times 10^{-5}$	$3·2 \pm 1·6 \times 10^{-7}$	31
Spiperone	$1·5 \pm 0·2 \times 10^{-5}$	$4·0 \pm 1·3 \times 10^{-10}$	37500
Haloperidol	$1·8 \pm 0·2 \times 10^{-5}$	$3·2 \pm 1·3 \times 10^{-9}$	5625
Azaperone	$5·4 \pm 0·6 \times 10^{-5}$	$2·0 \pm 1·6 \times 10^{-7}$	270
Acetylcholine	$7·5 \times 10^{-4}$	—	—
Hexamethonium	$>10^{-4}$	—	—
Nicotine	$>10^{-4}$	—	—
(+)-Tubocurarine	$>10^{-4}$	—	—

* Ratio [^{3}H] dexetimide/[^{3}H]Haloperidol.

nicotinic drugs were inactive whereas muscarinic antagonists like dexetimide, atropine or isopropamide had a very high affinity in the nanomolar range. In agreement with these results, dexetimide was found to be the most potent antimuscarinic agent when tested on the acetylcholine receptor in cultured nerve cells (Richelson & Divinetz-Romero, 1977). Of the other drugs tested, clozapine, thioridazine and perlapine displayed a relatively high affinity for the cholinergic receptors when compared with their affinity for the [^{3}H]haloperidol sites. Clozapine, for instance, had more affinity for the muscarinic than for the neuroleptic receptors. By contrast, pimozide, which is known to have relatively low incidence of extrapyramidal side-effects (cf. Ayd, 1971), displayed a low affinity for the cholinergic receptors but a very high one for the dopamine receptors when tested in the [^{3}H]haloperidol binding assay.

As shown in Table 1, the ratio dexetimide: haloperidol binding was much lower for clozapine than for pimozide and the butyrophenones. Therefore the relative potency of neuroleptics as antimuscarinic agents cannot be evaluated without taking into account their affinity for the dopamine receptors. For instance, the high ratios of pimozide, haloperidol and spiperone in dexetimide: haloperidol binding makes it unlikely that these drugs would have pharmacological or clinical antimuscarinic properties. Since the IC50 values for pimozide ($4·5 \times 10^{-6}$M) in the dexetimide binding assay markedly differed from that reported by Yamamura &

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Table 2. Displacement by drugs of labelled dexetimide in different brain areas. [^{3}H[Dexetimide (5 μg kg^{-1}, i.v.) was injected into rats. One h later unlabelled drug (mg kg^{-1}, dexetimide 0·63, clozapine 20, thioridazine 20, pimozide 0·63) was given intravenously then 3 h later, the radioactivity was measured in the different areas. The values are the means of six determinations ± s.e.m. Significant differences from control values are given by *P< 0·01 †P< 0·001 (P: Student's t-test).

Brain area	[^{3}H] Dexetimide pgmg^{-1} Cont.	Dexet.	Cloz.	Thior.	Pim.
Striatum	4·7 ±0·2	1·17† ±0·03	1·54† ±0·07	3·52* ±0·08	3·9 ±0·4
Frontal cortex	4·63 ±0·02	1·13† ±0·04	1·18† ±0·05	2·58 ±0·04†	4·21 ±0·04
Nucleus accumbens	4·6 ±0·4	1·63† ±0·05	1·8† ±0·2	4·13 ±0·2	5·4 ±0·3
Tuberculum olfactorium	3·6 ±0·1	1·08† ±0·05	1·28† ±0·02	2·98 ±0·06*	3·94 ±0·07
Hippocampus	3·5 ±0·2	0 97† ±0·08	0·95† ±0·02	2·0† ±0·1	3·2 ±0·1
Medulla oblongata	1·34 ±0·06	0·66† ±0·02	0·71† ±0·01	0 94† ±0·02	1·26 ±0·01
Cerebellum	0·65 ±0·05	0·51 ±0·02	0·51 ±0·02	0·62 ±0·01	0·69 ±0·01

others (1976) who tested it in the [^{3}H]quinuclidinyl benzylate binding assay (3·5 × 10^{-8}M), we made control experiments to prevent the difficulties inherent in obtaining pimozide in solution. When the drug was dissolved either in ethanol or in water with addition of lactic acid or tartaric acid, IC50 values identical to that reported in Table 1 were obtained.

To confirm these results, displacement experiments were made *in vivo* using [^{3}H]dexetimide as ligand. Table 2 shows that [^{3}H]dexetimide was specifically displaced by 0·63 mg kg^{-1} of unlabelled dexetimide namely in the brain regions containing a high amount of muscarinic receptors but not in the cerebellum. The same dose of (−)-benzetimide did not cause any displacement, thus proving the sterospecific nature of such *in vivo* binding (unpublished results). Clozapine, thioridazine and pimozide were also tested in this system. A dose of 0·63 mg kg^{-1} of pimozide may be considered as a very high dose since it is equal to about 13 times the subcutaneous ED50 value in the apomorphine tests in rats (Niemegeers, Lenaerts & others, 1977). When injected intravenously at this dose, pimozide did not change the disposition of [^{3}H]dexetimide in various brain areas (Table 2) even though all the neuroleptic receptors are occupied and the elevation in homovanillic acid is maximal (unpublished results). Clozapine and thioridazine were injected at 20 mg kg^{-1}, a dose which is respectively 3 times the oral and 5 times the subcutaneous EC50 value in the apomorphine test (Leysen, Niemegeers & others, 1977b) and therefore relatively much lower than 0·63 mg kg^{-1} of pimozide. In these conditions, clozapine and thioridazine displaced labelled dexetimide in the rat brain. Similar displacement was obtained using 0·63 mg kg^{-1} of dexetimide. Therefore the foregoing experiments largely confirm the results obtained in the *in vitro* binding assays. From these data it may be concluded that clozapine and thioridazine belong to that class of drugs endowed with potent antimuscarinic properties. This, in addition to their low affinity for neuroleptic receptors, could explain why these drugs are relatively weak antipsychotics. Indeed, clinical data have indicated that antiacetylcholine drugs decrease the therapeutic efficacy of neuroleptics (Sing & Smith, 1973) moreover the antiacetylcholine drugs antagonized the inhibition of brain stimulation and the elevation of homovanillic acid content produced by neuroleptics (cf. Wauquier & Niemegeers, 1975). Rather than an advantage, the antimuscarinic property of an antipsychotic may be considered as a drawback by making the drug a less potent antipsychotic.

From the present results, it may also be concluded that pimozide does not possess antimuscarinic properties. Hence the low incidence of induced extrapyramidal side-effects seen with pimozide must be attributed to other factors, for instance, its relatively *slow onset* of action. This has been explained by a balance between aspecific and specific binding. (Leysen & Laduron, 1977). Indeed, the retention of pimozide at aspecific sites and its slow dissociation therefrom could modulate the free drug concentration in the vicinity of the receptors in such a way that these are only slowly occupied, allowing an easier adaptation after post-synaptic blockage.

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Lithium inhibition of the adenosine-induced increase of adenylate cyclase activity

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Adenosine has been shown to stimulate accumulation of cyclic (c) AMP in brain tissue (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang, Shimizu & Daly, 1971; Schultz & Daly, 1973). Although the exact mechanism of adenosine action is unknown this molecule most likely interacts directly with adenylate cyclase (Daly, 1976). In brain tissue slices adenosine appears to potentiate the effects of some neurohormones on formation of cAMP (Sattin & Rall, 1967; Rall & Sattin, 1970; Huang & others, 1971, Schultz & Daly, 1973; Daly, 1976). The precise function of adenosine in the cns is still unclear although the available evidence would suggest a general modulatory role in regulating neural transmission in several different brain areas (Daly, 1976).

Lithium, an effective drug in the treatment of mania (Schou & Thomsen, 1975), is known to inhibit a number of adenylate cyclases both within the cns (Dousa & Hechter, 1970; Forn & Valdecasas, 1971) and outside it (Wolff, Berens & Jones, 1970; Geisler, Wraae & Olesen, 1972; Ebstein, Belmaker & others, 1976). However, its inhibitory effect on this enzyme is not ubiquitous since some hormone-specific adenylate cyclases are not inhibited (Ebstein, Kara & Belmaker, 1977; Olesen, Jensen & Thomsen, 1974). The increasing importance attributed to adenosine in central neurotransmission prompted us to examine the effect of lithium on the adenosine-stimulated accumulation of cAMP in a crude synaptosome preparation from guinea-pig cortex and caudate nucleus. There has been no previous report of a specific inhibitor of the adenosine-induced stimulation of cAMP accumulation.

A crude vesicular synaptosome preparation was prepared by a modification of the method of Chasin, Mamrek & Samaniego (1974). The tissue was homogenized in a glass Teflon homogenizer in 10 volumes Krebs Ringer phosphate (KRP) medium (including glucose). The homogenate was gassed with 5% CO_2 in oxygen for 20 s and incubated in the presence of [^{3}H]adenine (5μCi/100 mg wet weight tissue; Amersham, 5Ci m mol^{-1}). After 40 min at 37° in a shaking water bath, the homogenate was centrifuged at 900 *g* for 15 min and washed twice with an equal volume of KRP. After the second wash the pellet was resuspended in the original volume of KRP and divided into separate vials and incubated after gassing for an additional 15 min at 37° in the simultaneous presence and absence of adenosine (10μM), noradrenaline (100μM) or lithium (1, 2 and 5mM). The reaction was stopped with 100 μl 1N HCl. cAMP was purified by Dowex 50 chromatography and precipitation with $ZnSO_4$ and Na_2CO_3 as described by Krishna, Weiss & Brodie, (1968). Samples were counted in a Packard Tri-Carb scintillation counter.

Basal formation of cAMP was calculated as the amount of radioactive cAMP formed from radioactive precursor in the absence of adenosine, noradenaline and lithium. Lithium (up to 5·0mM) had no effect on basal activity from either cortex or caudate nucleus. Addition of adenosine or noradrenaline caused a rise in the radioactive cAMP formation, from which basal activity was subtracted to yield the specific stimulus-induced rise in cAMP formation. On each experimental day the rise in cAMP formation was determined in quadruplicate in the absence of lithium, and then in duplicate at each of four lithium concentrations. The percentage inhibition caused by lithium was calculated by comparing the rise in cAMP formation with adenosine or noradrenaline alone to the rises in the presence of various lithium concentrations. The average stimulation due to adenosine in the cortex was 79 $\pm$ 10% and in the caudate nucleus was 46 $\pm$ 9%.

Fig. 1 shows the effect of different concentrations of lithium on the adenosine-induced accumulation of cAMP in guinea pig-cortex and caudate nucleus. Starting at 1mM, it inhibited the adenosine-stimulated

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