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Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system

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In gallamine-immobilized cats, the caudate nucleus and the nucleus accumbens septi were perfused by means of a push-pull cannula and dopamine was measured in the perfusate. Chlorpromazine (10 mg kg^{-1}) and clozapine (20 mg kg^{-1}), administered intravenously, enhanced the release of dopamine. The effect of chlorpromazine was similar in both regions whereas that of clozapine was more pronounced in the nucleus accumbens than in the caudate nucleus. Furthermore, in the rat, sulpiride, clozapine and thioridazine increased the homovanillic acid concentration in striatum and limbic system to a similar extent. However, following probenecid administration, the net effect of these drugs on homovanillic acid accumulation was more marked in the limbic system than in the striatum whereas haloperidol and chlorpromazine had a similar effect in the two regions. It is concluded that, in contrast to haloperidol and chlorpromazine, sulpiride, clozapine and thioridazine may preferentially affect the limbic dopaminergic transmission. This possibly accounts for the fact that sulpiride, clozapine and thioridazine display an antipsychotic action and yet cause less extrapyramidal side effects than haloperidol and chlorpromazine.

The extrapyramidal side effects of neuroleptic drugs probably originate from the blockade of dopamine receptors in the striatum (cf. Hornykiewicz, 1972). The antipsychotic action of these agents, however, is possibly not connected with the impairment of the nigrostriatal dopamine transmission. In fact, sulpiride (Borenstein, Champion & others, 1969; Haase, Florn & Ulrich, 1974; Tagliamonte, de Montis & others, 1975), clozapine (Bürki, Eichenberger & others, 1975) and thioridazine (Cole & Clyde, 1961), which are claimed to display antipyschotic properties (1) do not cause marked extrapyramidal side effects in either man or animals and (2) are by far less potent than classical neuroleptic drugs (*e.g.* chlorpromazine and haloperidol) in enhancing striatal dopamine turnover which is thought to be triggered by the blockade of dopamine receptors (*vide infra*).

A preferential impairment of dopamine transmission in the limbic system by sulpiride, clozapine and thioridazine might account for their antipsychotic activity. We have therefore investigated the effects of these drugs on the dopamine release in both extrapyramidal and limbic system and compared their action to that of chlorpromazine and haloperidol.

MATERIALS AND METHODS

Two types of experiment were performed:

In the gallamine-immobilized cat (2·5–3 kg, either sex), a push-pull cannula (2 parallel cannulae welded together; outer diameter of each cannula, 0·20 mm) was stereotaxically implanted in the head of the caudate nucleus (A = 16, L = 3·8, H = + 4·5, Snider & Niemer, 1961) or in the nucleus accumbens septi (A = 15·5, L = 2·5, H = −2, Snider & Niemer, 1961) which were perfused with physiological Ringer solution (30 μl min^{-1}), as previously described (Stadler, Lloyd & others, 1973; Stadler, Lloyd & Bartholini, 1974; Lloyd & Bartholini, 1975). Dopamine was measured by a radioenzymatic essay (Coyle & Henry, 1973) in samples of the perfusate collected for 1 h before, and 3 h after intravenous administration of saline, chlorpromazine (10 mg kg^{-1}) or clozapine (20 mg kg^{-1}). The animals were kept normothermic throughout the perfusion at the end of which they were killed by an overdose of pentobarbitone. The localization of the cannula was verified histologically.

Male albino rats (strain Füllinsdorf, SPF bred), 180–200 g, were injected subcutaneously with (in mg kg^{-1}) chlorpromazine (0·25 and 0·5), haloperidol (0·025), thioridazine (3), clozapine (15) or sulpiride (20), either alone or 15 min after administration of probenecid (200 mg kg^{-1}, i.p.). The animals were decapitated 105 min after injection of the neuroleptic drugs. Other rats received various doses of probenecid alone and were killed at different times (see Fig. 1). In another series of rats, α-methyl-*p*-tyrosine methylester (200 mg kg^{-1}) was injected 1, 2 and 4 h before death. Rats injected with saline served as controls. The treated animals were kept normothermic in a heated box (30°). After decapitation, the

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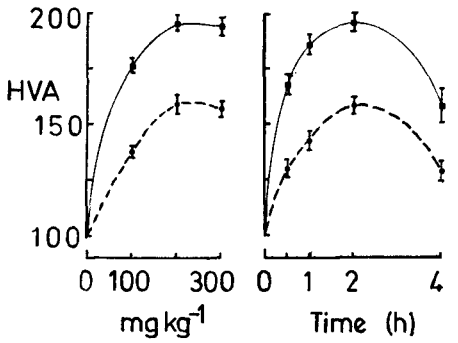

FIG. 1. Effect of probenecid on homovanillic acid (HVA) concentrations in striatum (– –) and limbic (—) system of the rat. Accumulation of HVA: left, 2 h after various doses of probenecid; right, at different times after 200 mg kg^{-1} of the compound. Probenecid was administered intraperitoneally. The values are expressed in percent of controls (= 100%) and represent averages with s.e.m. of results from 6 experiments, each of them consisting of duplicate determinations per dose or per time. Absolute control values: limbic system 165 ± 12, striatum 748 ± 21 ng g^{-1}. All of the values: $P < 0.01$ *vs* corresponding control.

brain was rapidly removed and put on an ice-cold glass plate. The brain ventricles were opened and the corpora striata dissected avoiding severance of the septum. A section was then performed through the remaining part of the brain from the superior colliculi to the optic chiasma. The dopamine-rich limbic areas are contained in the brain regions rostral to this section which will be referred to as the limbic system. The corpora striata as well as the limbic system from two rats were pooled and frozen in dry ice-cold heptane for subsequent measurement of dopamine (Shellenberger & Gordon, 1971) and extraction (Murphy, Robinson & Sharman, 1969) and fluorimetric determination (Andén, Roos & Werdinius, 1963) of homovanillic acid (HVA). Statistical analysis of the results was performed by *t*-test after one or two ways analysis of variance.

Drug solutions

Clozapine and thioridazine were dissolved in 0·1 N HCl. Water solutions of chlorpromazine HCl (Largactil, Rhone-Poulenc), sulpiride HCl (Dogmatil Delagrange) and haloperidol (Jansen) were acidified with HCl (final concentration, 0·1 N). All of the solutions were adjusted at pH 7·0 with 0·2 and 0·1 N NaOH. The following volumes were injected into cats: 1 ml kg^{-1}, i.v.; 2·5 μl g^{-1}, subcutaneously.

Probenecid was dissolved in 0·1 N NaOH and the solution adjusted at pH 7·5 with 0·2 and 0·1 N HCl. A volume of 1 ml kg^{-1} was injected intraperitoneally.

RESULTS

Release of dopamine in the caudate nucleus and nucleus accumbens of the cat

After intravenous administration of chlorpromazine (10 mg kg^{-1}) or clozapine (20 mg kg^{-1}), the output of dopamine from both caudate nucleus and nucleus accumbens septi was markedly increased for 3 h compared to the pre-injection period and to the corresponding post-injection period in saline-treated animals (Table 1). Chlorpromazine enhanced the release of dopamine in the caudate nucleus and the nucleus accumbens septi to a similar extent. In contrast, the clozapine-induced increase in dopamine liberation was more marked in the nucleus accumbens

Table 1. *Release of dopamine into the perfusate of the caudate nucleus and the nucleus accumbens of the cat.*

Treatment (mg kg^{-1}, i.v.)	Hours of perfusion: pre-inj. 0–1	post-inj.[a] 1–2	2–3	3–4	pre-inj. 0–1	post-inj.[a] 1–2	2–3	3–4
	Caudate nucleus pg dopamine				Nucleus accumbens pg dopamine			
Saline	150 ±12	160 ±3	152 ±21	162 ±9	136 ±27	128 ±13	146 ±16	136 ±4
Chlorpromazine (10)	143 ±16	284 ±28	405 ±67	514 ±83	161 ±17	264 ±30	273 ±40	364 ±52
Clozapine (20)	164 ±20	235 ±21	210 ±16	241 ±38	142 ±39	754 ±83	370 ±41	311 ±29

[a] Values of the post-injection period *vs* those of the pre-injection period in both regions: saline, $P > 0.05$; chlorpromazine and clozapine, $P < 0.01$; n = 4–8 cats per group. Results are mean ± s.e.m.

than in the caudate nucleus. The percentage enhancement of the amounts of dopamine released during 3 h after clozapine was lower in the caudate nucleus and higher in the nucleus accumbens than that caused by chlorpromazine (Table 2).

Table. 2. *Release of dopamine in the 3 h perfusate of the caudate nucleus and nucleus accumbens of the cat.*

Treatment (mg kg^{-1}, i.v.)	Overall post-injection release: Caudate nucleus pg/3h[a]	%	Nucleus accumbens pg/3h[a]	%
Saline	474±16	100±3·5	410±9	100±2·3
Chlorpromazine (10)	1203±51	257±13·0[b]	901±39	220±10·6[c]
Clozapine (20)	676±24	143±6·3[d]	1435±43	350±12·0[e]

[a] Values calculated from those of Table 1. All of the values, $P < 0.01$ *vs* corresponding controls (saline treated animals). b *vs* c, $P > 0.05$. d *vs* e, $P < 0.01$.

Accumulation of HVA in the extrapyramidal and limbic areas of the rat

In both striatum and limbic system of the rat, the concentration of HVA was increased after administration of 100–300 mg kg^{-1} probenecid (i.p.).

The percentage rise of the acid concentration was more marked in the limbic system than in the striatum. In both areas, 200 mg kg^{-1} of the compound had a maximal effect 2 h after administration. The HVA in the striatum and the limbic system reached a peak at about the same time (Fig. 1).

Sulpiride, clozapine and thioridazine caused a similar percentage increase of the HVA concentration in the limbic system and in the striatum whereas haloperidol and chlorpromazine (at both doses) enhanced the acid concentration more in the striatum than in the limbic system (Table 3). However, following probenecid administration, the net percentage rise caused by sulpiride, clozapine or thioridazine (at equipotent doses when administered alone) was 2·6, 1·6 and 1·3 times more pronounced in the limbic system than in the striatum, respectively. The effect of haloperidol was similar in both regions and that of chlorpromazine (at both dose levels) appeared to be more marked in striatum than in limbic system: the difference, however, did not reach statistical significance (Table 3).

In rats administered α-methyl-*p*-tyrosine, a pronounced decrease in the dopamine content occurred in both striatum and limbic system. In the latter region, the dopamine disappearance was significantly more marked during 4 h than in the former (Table 4).

DISCUSSION

In the experiments on cats with implanted push-pull cannulae, chlorpromazine and clozapine enhance the dopamine liberation in the caudate nucleus and nucleus accumbens septi. Together with the unchanged concentration of dopamine in these perfused regions following neuroleptic drugs (Lloyd, Stadler & Bartholini, 1973), these results indicate that the turnover of the amine is accelerated. This effect of neuroleptic agents probably results from the feedback activation of dopamine neurons triggered by the blockade of dopamine receptors (cf. Carlsson, 1974). A cholinergic mechanism appears to be involved in these events since antiacetylcholine

Table 3. *Effect of neuroleptic drugs, probenecid (200 mg kg^{-1}) and their combination on the homovanillic acid concentrations in limbic system (LS) and striatum (S) of the rat*[a]. The neuroleptic agents and probenecid were administered 105 and 120 min before death, respectively.

Brain regions (n)	Controls ng g^{-1}	Controls %	Neuroleptic (mg kg^{-1}) ng g^{-1}	Neuroleptic (mg kg^{-1}) % of controls[b]	A Probenecid ng g^{-1}	A Probenecid % of controls[c]	B Probenecid + neuroleptic ng g^{-1}	B Probenecid + neuroleptic % of controls	C Net neuroleptic effect (B-A) %	C in LS / C in S
			Sulpiride (20)							
LS (14)	171±10	100±5·8	269±22	157·7±9·3	349±17	208·8±10·6	480±20	289·6±13·9[d]	81·0±8·4[d]	2·6
S	764±33	100±4·3	1167±93	152·9±10·1	1209±50	159·8±6·3	1435±53	190·9±8·4	31·0±7·4	
			Clozapine (15)							
LS (12)	149±6	100±4	265±11	182·0±8·2	315±12	226·8±8·5	434±24	318·8±8·6[d]	87·0±8·2[d]	1·6
S	695±28	100±4	1261±41	186·8±4·9	1127±49	177·3±8·1	1518±62	239·8±7·4	56·0±9·1	
			Thioridazine (3)							
LS (10)	158±7	100±4·4	204±10	129·4±6·7	289±13	183·1±10·1	372±18	236·8±13·4[d]	61·5±3·9[d]	1·3
S	742±37	100±5·0	1033±55	137·4±5·8	1099±41	147·6±8·7	1420±51	191·1±11·6	47·5±2·1	
			Haloperidol (0·025)							
LS (8)	159±7	100±4·4	295±15	186·5±11·2	313±18	197·8±12·3	530±44	332·6±23·9[e]	134·5±13·5[e]	0·97
S	704±49	100±7·0	1612±66	229·9±11·0	1121±62	160·0±10·2	2111±146	299·4±18·1	139·3±15·9	
			Chlorpromazine (0·25)							
LS (6)	181±5	100±2·8	240±9	132·4±4·3	406±26	224·2±13·6	469±21	258·5±8·4[e]	34·3±8·6[e]	0·68
S	803±22	100±2·7	1263±56	157·2±5·6	1349±86	168·3±10·6	1755±51	218·5±2·6	50·3±5·8	
			Chlorpromazine (0·5)							
LS (8)	175±5	100±2·9	299±25	170·1±12·2	376±13	216·0±7·3	527±31	301·4±14·1[e]	85·3±23·4[e]	0·82
S	780±27	100±3·5	1630±133	207·2±12·3	1203±52	154·9±7·2	2019±85	259·2±8·6	104·3±12·1	

[a] The results are averages with s.e.m. of individual absolute and percentage values from 6–14 experiments (n). Each individual percentage was calculated from experiments made on the same day; in each experiment, the treatment of the 4 groups of rats (controls, neuroleptic drug, probenecid, probenecid plus neuroleptic drug) as well as the subsequent determinations were carried out in parallel.

[b] All of the values: $P < 0·01$ *vs* corresponding controls. LS *vs* S $P > 0·05$ for sulpiride, clozapine and thioridazine; $P < 0·01$ for haloperidol and chlorpromazine (both doses).

[c] All of the values: $P < 0·01$ *vs* corresponding control; LS *vs* S $P < 0·01$.

[d] LS *vs* S $P < 0·001$.

[e] LS *vs* S $P > 0·05$.

Table 4. *Effect of α-methyl-p-tyrosine (αMT, 200 mg kg^{-1}, i.p.) on dopamine concentrations in striatum and limbic system of the rat.*

Treatment		Dopamine[a] Striatum μg g^{-1}	Striatum %	Limbic system μg g^{-1}	Limbic system %
Saline		8·78±0·29	100±3·4	1·84±0·02	100±1·5
αMT±	1 h	6·36±0·23	72·4±2·5[b]	1·14±0·04	62·1±2·2
	2 h	4·69±0·20	53·4±2·1[b]	0·82±0·02	45·1±1·3
	4 h	2·81±0·11	32·1±1·2[b]	0·46±0·00	25·5±0·3

[a] Averages with s.e.m. of results from 4 experiments, each of them consisting of duplicate determinations per group.
[b] $P<0·01$ *vs* the value in the limbic system at the corresponding time.

drugs diminish the neuroleptic-induced activation of dopaminergic neurons whereas acetylcholine-like agents enhance the dopamine turnover (Andén, 1974; Bartholini, Keller & Pletscher, 1975).

The present results also show that 20 mg kg^{-1} clozapine enhance the striatal dopamine liberation less than 10 mg kg^{-1} chlorpromazine. This effect cannot be fully explained by the antiacetylcholine properties of clozapine which may diminish the feed-back activation of extrapyramidal dopamine neurons (Andén, & Stock, 1973). In fact, in contrast to antiacetylcholine compounds—which increase the striatal acetylcholine output, possibly due to a feed-back activation of cholinergic neurons (in preparation)—clozapine enhances the acetylcholine release only at the dose of 50 mg kg^{-1} (i.v.) (Stadler & others, 1974). This suggests that clozapine does not exert a marked antiacetylcholine action within the striatum. In addition, clozapine is by far less potent than chlorpromazine in enhancing the striatal acetylcholine release (Stadler & others, 1974), an effect which is the consequence of the dopamine receptor blockade (Stadler & others, 1973; Bartholini, Stadler & others, 1976). Therefore, as previously postulated (Bartholini, Haefely & others, 1972), the less marked activation of the nigrostriatal dopamine neurons by clozapine is likely to result from a less pronounced blockade of dopamine receptors than that caused by chlorpromazine.

Although clozapine seems to be a less potent receptor blocking agent in the striatum, compared with chlorpromazine it causes a more pronounced rise of dopamine release in the nucleus accumbens septi than in the caudate nucleus of the cat. This is in agreement with findings in the rabbit (Andén & Stock, 1973). In contrast, previous data as well as the present results from the rat show that the accumulation of HVA caused by clozapine (Bartholini & others, 1972), sulpiride and thioridazine, is similar in the striatum and the limbic structures. However, in probenecid-treated animals in which the transport of HVA is blocked, sulpiride, clozapine and thioridazine cause a more marked percentage accumulation of the acid in limbic system than in striatum. The preferential effect of these compounds (at doses that are equipotent in the absence of probenecid) decreases in the following order: sulpiride > clozapine > thioridazine. The peculiar pattern of action of these drugs, compared with that of haloperidol and chlorpromazine (which cause a similar accumulation of HVA in the two areas), could be accounted for by several mechanisms. For instance, it may be assumed that in the striatum probenecid produces only a partial blockade of the HVA clearance, or that its action is shorter than in the limbic system. However, these explanations are unlikely since, in the two areas, the accumulation appears to proceed at a comparable rate, attaining maximal concentration 2 h after administration of 200 mg kg^{-1} probenecid. Alternatively, a more efficient elimination of HVA into the cerebrospinal fluid from the striatum than from the limbic system—due to the proximity of the former structure to the ventricular space—could explain the preferential accumulation of the acid in limbic areas after sulpiride, clozapine, and thioridazine. This mechanism, however, should operate also following administration of haloperidol and chlorpromazine which, on the contrary, cause a similar accumulation of HVA in both structures. Furthermore, a faster elimination of sulpiride, clozapine and thioridazine from the striatum than from the limbic system—which could lead to a less marked effect of these drugs in the former structure—is also unlikely. Thus, based on determination of water: organic solvents partition coefficients, the apparent liposolubility of the five neuroleptic drugs examined is similar (Kyburz, personal communication). Finally, the accumulation of sulpiride, clozapine and thioridiazine into the brain could be affected by probenecid in a manner different from that of haloperidol and chlorpromazine. This hypothesis, however, does not seem to receive support from any known experimental result.

The preferential accumulation of HVA in the limbic system of the probenecid-treated rat following sulpiride, clozapine and thioridazine is possibly connected to a more pronounced activation of dopamine neurons in this region than in the striatum. Supporting evidence for this view is provided by the results obtained with clozapine in cats, which directly show that the liberation of dopamine is more markedly enhanced in the nucleus accumbens than in the

caudate nucleus. The peculiar biochemical profile of these drugs is possibly linked to a stronger blocking action on limbic than on striatal dopamine receptors.

It is not clear why, in the rat, sulpiride, clozapine and thioridazine, administered alone, cause an accumulation of HVA in limbic areas comparable to that in striatum, while after probenecid administration their effect is more marked in the limbic systems. It can be postulated that the transport mechanism of the acid is more efficient in limbic areas than in the striatum. Thus, only after blockade of the HVA transport, does the preferential effect of these drugs appear. However, this hypothesis remains to be proved.

The mechanism by which sulpiride, clozapine and thioridazine possibly cause a preferential activation of limbic dopamine turnover is far from being explained. It may be connected with a greater accumulation of these drugs in limbic than in extrapyramidal structures, possibly accounted for by a difference in the blood-brain barrier of the two areas. Alternatively, these compounds may possess, as compared to haloperidol and chlorpromazine, a stronger affinity for limbic than for striatal dopamine receptors.

The fact that probenecid, given alone, causes a more marked accumulation of HVA in the limbic system than in the striatum suggests that, under physiological conditions, the dopamine turnover is faster in limbic structures. This is confirmed by the α-methyl-*p*-tyrosine-induced disappearance of dopamine which is more pronounced in limbic areas than in striatum. However, the possible relation between the difference in dopamine turnover rate and that in the action mechanism of neuroleptic drugs requires further studies.

In conclusion, the present results indicate that the neuroleptic drugs sulpiride, clozapine and thioridazine are weak dopamine receptors blocking agents; their action, however, appears to be more pronounced in the limbic system than in the striatum. In contrast, haloperidol and chlorpromazine possess stronger dopamine receptor blocking properties which however result in a similar effect in both structures. The possibility that sulpiride, clozapine and thioridazine more markedly affect limbic than striatal dopamine receptors may account for the fact that these drugs display an antipyschotic action and yet cause less extrapyramidal side effects than haloperidol and chlorpromazine.

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