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DOPAMINERGIC EFFECTS OF BUSPIRONE, A NOVEL ANXIOLYTIC AGENT

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Abstract—The novel anxiolytic drug buspirone raised striatal levels of the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) 1 hr after oral administration. This effect was dose-dependent with a peak at 60 min. No changes were observed in the levels of 3-methoxytyramine (3MT), the extraneuronal metabolite of dopamine. Noradrenaline, serotonin and its metabolite 5-hydroxyindoleacetic acid (5HIAA) were not affected. Buspirone displaced [^{3}H]spiroperidol from striatal binding sites, with an IC_{50} (1.8×10^{-7} M), comparable to that of clozapine (IC_{50} = 1.4×10^{-7} M) but considerably lower than that of haloperidol (4.7×10^{-9} M). Buspirone was only a weak inhibitor of dopamine-stimulated adenyl cyclase. Buspirone was not active on the binding of trifluoperazine to calmodulin and did not modify calmodulin-induced activation of phosphodiesterase (PDE). Repeated administration of buspirone did not increase the number of DA receptors. These data show that, although buspirone has antidopaminergic activity, it can hardly be classified as a classic neuroleptic agent.

Buspirone is a novel anxiolytic agent [1–3] with a chemical structure different from the benzodiazepines [4, 5] (see Fig. 1). Pharmacologically buspirone is not an anticonvulsant [2] but it shares with benzodiazepines the property of being active in the treatment of anxiety [2, 6–8]. However buspirone has no affinity *in vitro* for brain benzodiazepine-binding sites or GABA receptors (Mennini *et al.*, personal communication). Previous work from this laboratory has shown that buspirone reduces acetylcholine (Ach) levels in the rat striatum and nucleus accumbens but not in other parts of the brain [9]. This contrasts with the increase of brain Ach induced by benzodiazepines [10] and cannot be explained on the basis of a direct effect of buspirone on the enzymes synthesizing or metabolizing Ach [9].

In view of the link between the dopaminergic and cholinergic systems in the striatum [11, 12], experiments were made to investigate the action of buspirone on dopamine (DA) in the rat brain, particularly in the striatal area. Since cataleptogenic neuroleptics such as haloperidol and non-cataleptogenic neuroleptics such as clozapine [13] reduce striatal Ach [14] by inhibiting dopaminergic receptors, they were utilized as reference drugs in evaluating the effect of buspirone.

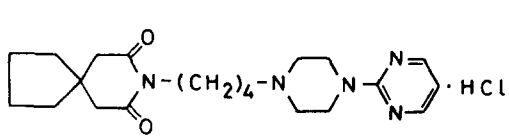

Fig. 1. Structural formula of buspirone hydrochloride.

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MATERIALS AND METHODS

CD-COBS rats (Charles River, Italy), housed under standard conditions with free access to food and water, were used.

For determination of DA and its metabolites, the animals were killed by microwave irradiation (1.3 W at 2.45 GHz for 4 sec) to rapidly inactivate catechol-*O*-methyltransferase (COMT). Striata were immediately dissected, frozen on dry ice and kept at −80° until biochemical assay. Homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were measured according to a method previously described [15]. DA was assayed as described by Keller *et al.* [16] and 3-methoxytyramine (3MT) according to Ponzio *et al.* [17]. For *in vitro* studies the animals were decapitated, the striata were rapidly dissected and analyzed for DA receptor and adenylate cyclase activity. The effect of drugs on [^{3}H]spiroperidol binding [New England Nuclear (35.9 Ci/mmole)] was determined in striatal membrane preparations according to Burt *et al.* [18] using 0.3 nM [^{3}H]spiroperidol. Briefly, rat striata were homogenized by sonification, and the crude membrane preparation was suspended to a final tissue concn of 3.5 mg wet wt/ml in 50 mM Tris–HCl (pH 7.1), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$. [^{3}H]Spiroperidol specific binding was defined as the difference in binding in the absence or presence of (+)-butaclamol (10^{-6} M). Adenylate cyclase activity was determined in striatal homogenate as described by Krishna *et al.* [19] using [8-^{14}C]adenosine triphosphate (ATP) as substrate [58 mCi/mmole (Amersham Radiochemical Center)] and measuring the 3′,5′-[^{14}C]cyclic adenosine monophosphate (cAMP) formed after 5 min incubation in the presence of DA at concns of 10^{-6}, 10^{-5} and

10^{-4} M, and chromatographic separation on Dowex 50 (H^+) columns. The competitive effect of buspirone on [^{3}H]trifluoperazine binding to calmodulin was determined according to the equilibrium dialysis procedure developed by Levin and Weiss [20] using purified beef brain calmodulin. Five hundred microlitres of calmodulin (20 μg/ml) were dialyzed for 18 hr at 4° in a bath containing 20 ml of 5 mM Tris–HCl (pH 7.0), 1 mM $MgCl_2$ and either 0.1 mM $CaCl_2$ or 0.3 mM ethylenebis(oxyethylenenitrilo)tetra-acetic acid (EGTA), [^{3}H]trifluoperazine (1 μM) and concns of non-labeled drugs ranging from 1 nM to 100 μM. At the end of the dialysis the [^{3}H]trifluoperazine bound was calculated as the difference between radioactivity found in the dialysis bag and that in the bath. Binding is expressed as calcium specific binding defined as the difference between the binding in the presence of Ca^{2+} and that in the presence of EGTA, Calmodulin-stimulated phosphodiesterase (PDE) activity was measured by the luciferin–luciferase method described by Weiss *et al.* [21] using 400 μM cAMP as substrate.

In chronic studies, one group of animals was treated orally for 25 days with buspirone (20 mg/kg body wt twice a day). Two other groups of animals received intraperitoneally either haloperidol (1 mg/kg body wt) or clozapine (20 mg/kg body wt) twice a day for the same length of time. The control groups received the vehicle. The animals were killed by decapitation 4 days after the last drug dose and binding of [^{3}H]spiroperidol was determined on fresh tissue. For the determination of equilibrium binding constants, specific binding data [i.e. the difference of binding in the absence and presence of (+)-butaclamol (10^{-6} M)] were transformed following Scatchard analysis.

RESULTS

Dose-response of buspirone on DA and its acid metabolites in the rat striatum

Buspirone was given orally at six doses, ranging from 2.5 to 80 mg/kg, and the biochemical determinations were performed 1 hr after the treatment; control animals received only saline. As shown in Table 1 striatal DA was only slightly reduced by the dose of 10 mg/kg, the decrease being maximal at the highest dose (80 mg/kg). Both acid metabolites of DA, DOPAC and HVA, were increased after buspirone administration. This effect was already statistically significant at the dose of 2.5 mg/kg and reached a pleateau around 10 mg/kg; further increases in the dose of buspirone did not further increase striatal DA acid metabolites.

In order to assess the specificity of this finding on DA with respect to other monoaminergic systems in parallel experiments buspirone was given at the oral dose of 20 mg/kg and striatal serotonin (5HT), 5-hydroxyindoleacetic (5HIAA) and noradrenaline (NA) were measured. When the animals were killed 1 hr after drug administration no significant differences could be observed (data not reported in detail). The fact that 5HIAA was not affected rules out the possibility that buspirone raises the levels of DOPAC and HVA by blocking the transport of acid metabolites with a mechanism similar to that exerted by probenecid [22].

Time-course of buspirone on DA and its metabolites in the rat striatum

Table 2 shows the effect of buspirone at the oral dose of 20 mg/kg on DA and its metabolites when the rats were killed at different times after drug administration (30, 60, 120 and 240 min).

DA levels were reduced but only up to 60 min after drug administration whereas the increase of both acid metabolites (DOPAC and HVA) lasted at least 2 hr.

In this experiment 3MT, the metabolite of DA formed after release of DA by COMT [23] was measured in the striatum. Unexpectedly 3MT was not affected by buspirone despite the major change in DA metabolism.

Comparison with haloperidol and clozapine on striatal DA metabolism

A direct experiment was performed to establish how buspirone compared with haloperidol, a cataleptogenic, and clozapine, a non-cataleptogenic neuroleptic, in affecting striatal DA metabolism. Drugs were given intraperitoneally at doses of 0.5 mg/kg (haloperidol) and 20 mg/kg (clozapine and buspirone) and the animals were killed 15, 30 and 60 min after drug injection. The doses of haloperidol and clozapine were selected on the basis of previous experience to obtain comparable increases in striatal

Table 1. Effect of buspirone on striatal DA, DOPAC and HVA

Buspirone (mg/kg)	Striatum (ng/g)		
	DA	DOPAC	HVA
0	8190 ± 220	761 ± 21	727 ± 40
2.5	8610 ± 240	1037 ± 95*	950 ± 90*
5	7420 ± 430	1255 ± 186*	1040 ± 141*
10	6260 ± 310*	1833 ± 161*	1777 ± 156*
20	6680 ± 180*	2281 ± 157*	1973 ± 77*
40	6580 ± 260*	2873 ± 191*	1975 ± 76*
80	5230 ± 420*	2149 ± 110*	1887 ± 183*

Buspirone was administered orally 1 hr before the rats were killed by microwave irradiation. Data, presented as ng/g, are the means ± S.E. of six assays. Statistical significance was determined by a modification of Duncan's multiple test [32].

* $P < 0.01$ vs controls.

Table 2. Time-course of buspirone effect on striatal DA and its metabolites

Striatal biochemical parameter	Minutes after buspirone*				
	0	30	60	120	240
3MT	10 ± 1	11 ± 1	12 ± 1	10 ± 1	—
DOPAC	850 ± 67	2389 ± 125†	2728 ± 326†	1482 ± 278†	1135 ± 79
HVA	537 ± 53	785 ± 37†	1230 ± 185	909 ± 50†	571 ± 89
DA	6930 ± 140	5600 ± 350†	5730 ± 230†	6100 ± 220	6690 ± 420

* Buspirone was administered at a dose of 20 mg/kg. Animals were killed by microwave irradiation at different times after drug administration. Data, presented as ng/g, are the means ± S.E. of six assays. Statistical significance was determined by a modification of Duncan's multiple test [32].

† $P < 0.01$.

HVA. As shown in Table 3 DA levels were not affected by any of the tested drugs. As regards buspirone the lack of effect reported in Tables 1 and 2 may be due to the relatively high variability observed in this group of controls. Striatal DOPAC and HVA were increased to a similar extent by the three drugs; the effect of clozapine appears to take longer than haloperidol and buspirone. A clear-cut difference was observed compared to 3MT; as expected, haloperidol raised the levels of this DA metabolite while buspirone and clozapine had no effect.

Effect of chronic buspirone treatment

In order to establish whether the effect of buspirone on DA and its acid metabolites persisted, rats were treated for 7 days with buspirone (20 mg/kg twice daily orally) or with saline. After 24 hr without treatment animals received saline or buspirone (20 mg/kg orally). Sixty minutes after this last treatment the animals were killed for biochemical determinations. As shown in Table 4, repeated treatment with buspirone significantly reduced the effect of a subsequent dose. However even after 7 days of treatment buspirone still raised the levels of striatal HVA and DOPAC.

Effect of buspirone on DA receptors

In order to clarify the reason for the increased metabolism of DA other experiments were performed to determine the effect of buspirone on DA receptors (Table 5). Buspirone displaced [^{3}H]spiroperidol from striatal membranes *in vitro*. The IC_{50} was 1.8×10^{-7} M compared to the effect of classic neuroleptics such as (+)-butaclamol (6.3×10^{-10} M) and haloperidol (4.7×10^{-9} M). Clozapine was effective at a concn of 1.4×10^{-7} M.

DA-stimulated adenyl cyclase was less sensitive to the action of buspirone which caused only 27% inhibition at a concn of 10^{-4} M.

Another binding site for neuroleptics is on calmodulin, a protein which activates cyclic nucleotide PDE [20]. Buspirone did not displace [^{3}H]trifluoperazine bound to calmodulin ($IC_{50} > 10^{-4}$ M) and did not block PDE activated by calmodulin ($IC_{50} > 10^{-4}$ M). Clozapine was active in both these cases (Table 5).

To see whether chronic buspirone affected the number of DA receptors in the striatum, the drug was given for 25 days at twice-daily oral doses of 20 mg/kg. Four days after drug discontinuation the number of [^{3}H]spiroperidol receptors (B_{max}) and the dissociation constant (K_d) were not significantly affected. In parallel experiments summarized in Table 6 clozapine was not effective, but haloperidol caused a statistically significant increase in the [^{3}H]spiroperidol B_{max}.

DISCUSSION

Previous studies have shown that buspirone reduces the levels of striatal Ach [9]. Since this decrease may be related to a blockade of post-synaptic dopaminergic receptors in the same brain area [11] it was of interest to establish whether buspirone acts on the dopaminergic system. The results of the present investigation are consistent with the possibility that the decrease of striatal Ach is at least partially mediated by an antidopaminergic activity of buspirone.

The evidence in favor of this activity is the dose-dependent increase of HVA and DOPAC, the acid metabolites of DA. This increase is not due to blockade of the transport of acid compounds as induced by probenecid [22], because if it were an increase of 5HIAA should also have been observed. Similarly it cannot be due to a direct releasing action of buspirone because *in vitro* no increase was seen in the release of [^{3}H]DA from prelabeled striatal synaptosomes (Riblet, personal communication).

The effect of buspirone on DA acid metabolites might however be explained by a feed-back mechanism due to a blockade of DA receptors not linked with adenyl cyclase. Indeed buspirone blocks by 50% the binding of [^{3}H]spiroperidol to striatal membranes at a concn of 1.8×10^{-7} M and slightly inhibits adenyl cyclase stimulated by DA at the concn of 10^{-4}.

Another explanation for the increased release of DOPAC and HVA may lie in buspirone's effect on DA presynaptic autoreceptors. However results in this regard are conflicting since buspirone may act as an agonist according to some authors [6, 24] and as an antagonist according to others [25]. Which of these effects predominates is difficult to establish at the present time. In fact *in vitro* studies with buspirone may not be representative of the *in vivo* situation considering that buspirone is extensively metabolized [26, 27], and it cannot be excluded at this time that an "active" metabolite may be present in the brain. Buspirone, like clozapine but unlike

Table 3. Effects of clozapine, haloperidol and buspirone on striatal DA metabolism

Striatal biochemical parameter	Controls	Minutes after drug injection								
		Clozapine			Haloperidol			Buspirone		
		15	30	60	15	30	60	15	30	60
DA	6257 ± 309	5749 ± 360	6297 ± 509	6327 ± 402	5727 ± 291	5698 ± 295	5429 ± 169	6216 ± 398	5539 ± 250	5749 ± 344
3MT	10.2 ± 1.1	11.7 ± 0.3	10.5 ± 0.9	8.9 ± 0.8	16.1 ± 1.3*	17 ± 1.1*	10 ± 1.1	11.2 ± 0.7	11.7 ± 1.1	10.4 ± 0.4
HVA	404 ± 45	456 ± 26	574 ± 39	794 ± 95*	511 ± 26	829 ± 61*	1152 ± 67*	637 ± 49	813 ± 82*	1096 ± 147*
DOPAC	872 ± 62	1356 ± 94*	1856 ± 180*	2688 ± 266	1828 ± 54*	2926 ± 270*	3358 ± 358*	2274 ± 182*	2642 ± 352*	2840 ± 422*

Clozapine (20 mg/kg), haloperidol (0.5 mg/kg) and buspirone (20 mg/kg) were injected intraperitoneally. Animals were killed by microwave irradiation at different times after drug administration. Data, presented as ng/g, are the means ± S.E. of 6 assays. Statistical significance was determined by Dunnett's test [32].

* $P < 0.01$.

Table 4. Effect of 7 days repeated and acute treatment with buspirone on the concns of DA, HVA and DOPAC in rat striatum

Chronic treatment Acute treatment	Vehicle + vehicle	Vehicle + buspirone	Buspirone + vehicle	Buspirone + buspirone
DA (ng/g) ± S.E.	8740 ± 644	6779 ± 753*	8088 ± 566	7352 ± 231
HVA (ng/g) ± S.E.	517 ± 37	1049 ± 104*†	409 ± 35	751 ± 51*†
DOPAC (ng/g) ± S.E.	504 ± 37	1263 ± 114*†	402 ± 38	923 ± 86*†

Repeated treatment: buspirone (20 mg/kg p.o.) or saline twice daily for 7 days; 24 hr withdrawal.
Acute treatment: buspirone (20 mg/kg p.o.) or saline 60 min before death.
Statistical significance was analyzed by two-way ANOVA [33] and Duncan's multiple test [32].

* $P < 0.01$ vs controls.

† $P < 0.01$ vs repeated buspirone.

Table 5. IC_{50} of buspirone and other drugs on *in vitro* dopaminergic receptors and on calmodulin

Drug	[^{3}H]Spiroperidol	DA-stimulated adenylate cyclase (DA = 10^{-4} M)	[^{3}H]Trifluoperazine Calmodulin	PDE Calmodulin
Buspirone	1.8×10^{-7} M	$> 10^{-4}$ M	$> 10^{-4}$ M	$>10^{-4}$ M
(+)-Butaclamol	6.3×10^{-10} M	1.8×10^{-7} M*	7.5×10^{-5} M†	—
Haloperidol	4.7×10^{-9} M	4.7×10^{-7} M	1.5×10^{-5} M	6×10^{-5} M†
Clozapine	1.4×10^{-7} M	5.5×10^{-7} M	3.0×10^{-5} M	8×10^{-5} M†

For details see Materials and Methods.
* From Miller *et al.* [34].
† From Levin and Weiss [20].

haloperidol (Table 3), did not change the level of striatal 3MT, the extraneuronal DA metabolite formed by the action of COMT [23], despite the changes observed in the DA metabolism. This lack of an effect on 3MT is difficult to explain and requires further investigation.

Whether the effect on the DA system helps explain the anticonflict activity of buspirone in laboratory animals [28, 29] and the anxiolytic activity in man [7, 8] is a matter of speculation. Buspirone is unable *in vitro* and *in vivo* to displace [^{3}H]diazepam from benzodiazepine-binding sites in rat brain (Mennini, this laboratory, unpublished data) and therefore it must have a mechanism of action different from that postulated for the benzodiazepines [30, 31]. The anxiolytic activity might possibly be related to the neuroleptic activity classically present in drugs showing antidopaminergic activity. However, as shown in the present study, buspirone differs from typical neuroleptic agents such as haloperidol because it does not raise striatal 3MT, it virtually does not block DA-mediated adenylate cyclase, it does not show affinity for calmodulin and it does not raise the number of DA receptors in the striatum after repeated treatment. In several respects buspirone resembles clozapine, an atypical neuroleptic [13], except that it does not inhibit central muscarinic receptors [9]. In addition clozapine differs from buspirone in that it is active on DA-stimulated adenylate cyclase and shows an affinity for calmodulin.

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Table 6.

Drug (mg/kg)	[^{3}H]Spiroperidol binding	
	B_{max} (fmoles/mg protein)	K_d (nM)
Control	397 ± 13	0.25 ± 0.025
Buspirone 20 × 2 (p.o.)	438 ± 10	0.25 ± 0.015
Control	369 ± 13	0.37 ± 0.001
Haloperidol 1 × 2 (i.p.)	440 ± 10*	0.36 ± 0.025
Control	224 ± 8	0.33 ± 0.04
Clozapine 20 × 2 (i.p.)	254 ± 6	0.36 ± 0.01

Specific binding of [^{3}H]spiroperidol (0.05–2 nM) was determined 4 days after the last treatment in a striatal membrane preparation from rats given vehicle or drugs twice a day for 25 days. Specific binding was considered as the difference in binding with and without (+)-butaclamol (10^{-6} M). To determine equilibrium binding constants, specific binding data were transformed following Scatchard analysis. The B_{max} and K_d values are the means ± S.E.M. of six independent Scatchard plots of two separate experiments, each established on pooled striatal membranes from three rats.
* $P < 0.02$ vs controls (Student's *t*-test) [35].

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