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Multiple and Complex Effects of Buspirone on Central Dopaminergic System

S. ALGERI, A. DE LUIGI, M. G. DE SIMONI, L. IMERI, M. MARCONI, S. NAVA, C. PEREGO AND G. SACCHETTI

Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea, 62, 20157 Milano, Italia

ALGERI, S., A. DE LUIGI, M. G. DE SIMONI, L. IMERI, M. MARCONI, S. NAVA, C. PEREGO AND G. SACCHETTI. *Multiple and complex effects of buspirone on central dopaminergic system.* PHARMACOL BIOCHEM BEHAV **29**(4) 823–826, 1988.—The effects of the anxiolytic drug buspirone and its metabolite 1-PP on the dopaminergic system were investigated. A single buspirone administration was found to decrease DA levels and increase its metabolite DOPAC in striatal samples. The levels of the other DA metabolite, 3MT, were unaffected; however its formation rate after inhibition of its metabolism, was found to be increased by buspirone. 1-PP did not affect either DOPAC or 3MT levels and formation. Striatal microdialysis showed that buspirone enhances DA release. *In vivo* voltammetry indicates that the increase of DA metabolism is identical in the two sampled dopaminergic areas, striatum and nucleus accumbens. On the basis of the results obtained *ex vivo* and *in vivo* the multiple effect of buspirone on dopaminergic system is discussed.

Buspirone Dopaminergic system Brain dialysis *In vivo* voltammetry

BUSPIRONE is a piperazinyl derivative provided with anxiolytic properties [5,6]. Differently from benzodiazepines, the classic anxiolytic drugs, it does not interact with the benzodiazepine-GABA receptor complex [9] but it shows high affinity for the spiperone binding site [3].

As it does not inhibit the dopamine (DA)-stimulated adenylate cyclase, as normally the D_1 antagonists do, it may be inferred that this drug is a D_2 antagonist. As the other D_2 antagonists, buspirone increases the concentration of DA acid metabolites, HVA and still more that of DOPAC. In contrast it does not increase the basal concentration of 3MT, a DA metabolite correlated with DA release, and surprisingly enough, for a DA receptor's antagonist, it decreases DA concentration. Therefore there is not clear biochemical evidence that this drug increases DA release similarly to neuroleptics acting on D_2 receptors. In an effort to get a better insight into the dopaminergic effects of this new drug, we present here the results obtained in a series of experiments performed using different methodological approaches, among which are *in vivo* techniques such as pulse voltammetry [4] and microdialysis [7].

METHOD

Animals

Male rats (CD-COBS, Charles River, Italy, 250–300 g) housed under standard conditions with free access to food and water were used.

Drugs

Buspirone and its metabolite 1-PP (Bristol-Myers, Evansville, IN) were dissolved in saline and injected intraperitoneally (IP).

Biochemical Determinations

For the determination of DA and its metabolites, rats were killed by focussed microwave irradiations. Striata were rapidly dissected and preserved at −80°C until biochemical assay. DA and its metabolites were assayed by electrochemical detection coupled with HPLC (LCED) according to the procedures developed in our laboratory [1,10].

In Vivo *Voltammetric Recordings*

Anesthetized rats (choral hydrate, 350 mg/kg, IP) were prepared for chronic recordings following the surgical procedure previously described [4]. Briefly, a working carbon fiber electrode (tip diameter 8 μm) was stereotaxically placed in caudate nucleus (A=9 mm, L=2.2 mm, V=4.5 mm down the brain surface) or nucleus accumbens (A=10.4 mm, L=1.55 mm, V=6.0 mm down the brain surface); a "t" screw and an Ag/AgCl wire served respectively as auxiliary and reference electrodes. All experiments were performed in conscious, freely moving rats after recovery from surgery. Measurements of extracellular DOPAC were performed using a polarograph (Biopulse, Tacussel, France) at the parameters described in a previous paper [4]. Voltammograms were recorded every 2 min and drug or saline was injected when the signal had been stable for at least 15 voltammograms. All data are expressed as percentages of a control value obtained by averaging the heights of the 6 peaks before drug injection.

TABLE 1

STRIATAL LEVELS OF DA AND ITS METABOLITES 30 MIN AFTER ADMINISTRATION OF BUSPIRONE AND 1-PP

	DA	3MT	DOPAC
Saline	6592 ± 786	20 ± 3	533 ± 90
Buspirone	3882 ± 343*	21 ± 3	1833 ± 91*
Saline		14 ± 1	428 ± 21
1-PP		13 ± 1	462 ± 31

Data are expressed as ng/g (mean ± SE of 5 determinations) *$p<0.01$ different from saline, Dunnett's test.

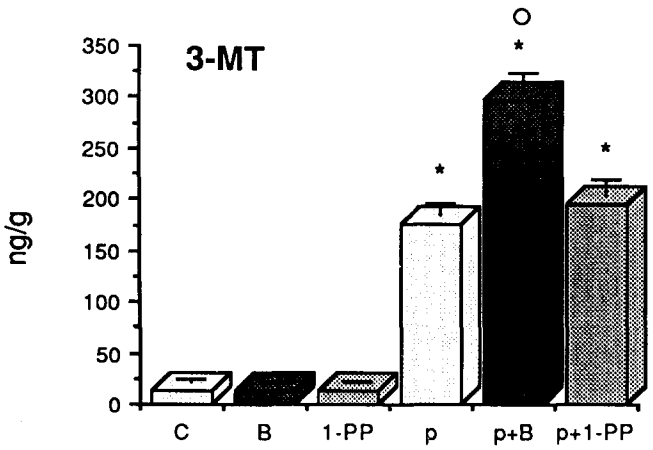

FIG. 1. 3MT levels in rats treated with pargyline (75 mg/kg IP) 5 min after buspirone (20 mg/kg IP) or 1-PP (10 mg/kg IP). Data are the mean±SE of 5 samples. C=saline; p=pargyline; B=buspirone. *Different from all the groups without pargyline; ○Different from *p* (significant interaction) by ANOVA and Tukey's test.

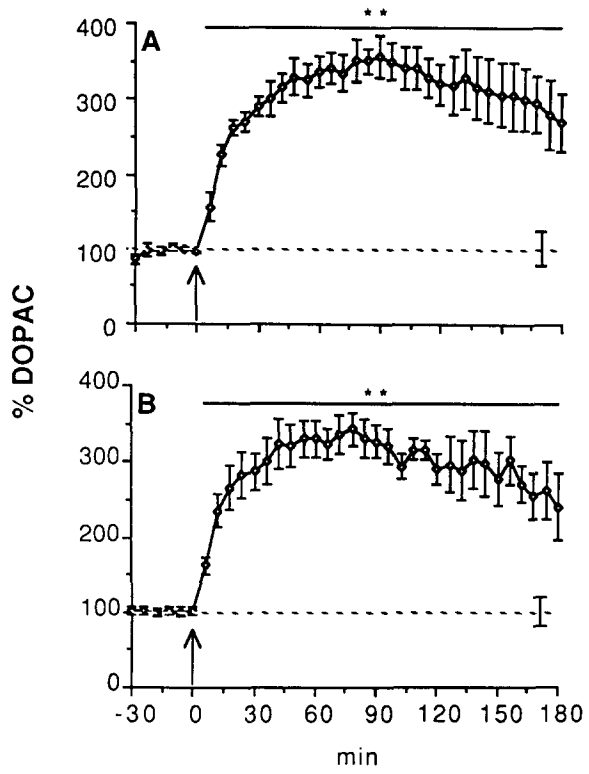

FIG. 2. Voltammetric determination of DOPAC levels in the caudate (A) and nucleus accumbens (B) of unanaesthetized freely moving rats: effect of buspirone, 10 mg/kg IP. The arrow indicates the time of injection. Data are the mean±SE of 3 rats and are expressed as explained in the Method section. The dotted line and the vertical bar represents the variation range of control recording (saline injection at arrow). **$p<0.01$ compared with $t=0$, ANOVA and Dunnett's test.

In Vivo *Microdialysis*

A polyacrylonitrile and sodium methallyl sulfonate copolymer dialysis tube (AN™, Hospal SpA; 0.25 outer diameter, with more than 15000 mW cutoff and with an *in vitro* determined recovery for DA and its metabolites of approximately 20%) was stereotaxically positioned in striata of chloral hydrate anesthetized rats as described by [7]. After 24 hours recovery the freely moving rats were used for the experiments. One end of the dialysis tube was connected through a polyethylene tubing to a syringe containing Kreb's solution and maintained to a constant flow of 2 μl/min by an infusion pump (Harvard Apparatus). Forty μl of perfusate was collected every 20 min in Eppendorf test tubes containing 10 μl of 0.1 N $HClO_4$. DA, HVA and DOPAC concentrations were determined by the above mentioned LCED procedures. Drug was injected after three hours of perfusion in order to reach constant basal values of DA and its metabolites.

RESULTS

Effects of Buspirone and 1-PP on DA and Da Metabolites Concentration in Striatum

The effect of buspirone administration (20 mg/kg IP, 30 min before sacrifice) on the striatal concentrations of DA, DOPAC and 3MT, are summarized in Table 1. The results obtained confirmed our previous results [3]: DA was decreased, DOPAC increased and 3MT unaffected. In another similar experiment, buspirone's effect on DA metabolism was compared with that of 1-PP, its main metabolite. Since 1-PP accumulates in the brain, reaching concentrations higher than the parent drug [5], it was injected at a lower dose (10 mg/kg IP). In contrast to buspirone it did not increase the levels of DA acid metabolite (Table 1). Both buspirone and 1-PP did not change basal 3MT levels, however the accumulation of this extraneuronal metabolite after block of its oxydative deamination by pargyline (75 mg/kg IP) is significantly enhanced in rats treated with busprione but not in rats treated with 1-PP (Fig. 1).

Buspirone Effect on Extraneuronal DOPAC Measured by In Vivo *Voltammetry*

Extraneuronal DOPAC was measured in striatum and nucleus accumbens of freely moving rats. The results obtained are summarized in the graphs of Fig. 2. In both dopaminergic areas, the levels of this metabolite started to increase immediately after the administration of busprione (10 mg/kg IP), reached a maximum after 60 min and remained at this high level for at least 3 hours. After 2 hours the DOPAC levels show a tendency to decrease back to normality.

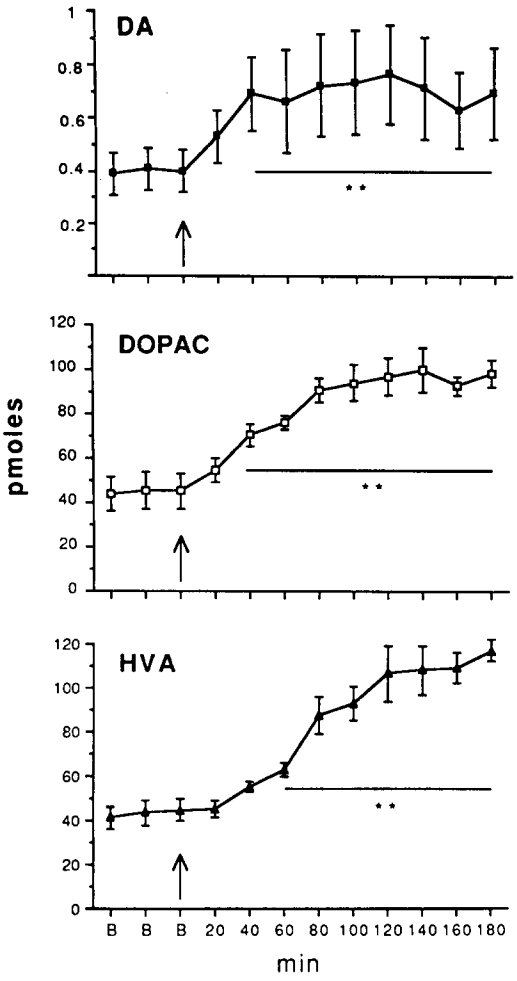

FIG. 3. DA, DOPAC and HVA levels in the dialysate of unanaesthetized rat striata: effect of buspirone, 10 mg/kg IP. The arrow indicates the time of injection. Data are expressed as pmoles in 20 min samples (40 μl of dialysate) and are the mean±SE of 4 rats. **$p<0.01$ compared with basal levels (B), ANOVA and Dunnett's test.

Buspirone Effect on DA Release From Striatal Neurons Measured by In Vivo *Microdialysis*

In order to verify directly the possibility that buspirone increases DA release, we have measured extraneuronal DA in freely moving rats through a microdialysis tube placed in the striata. The results of this experiment are presented in Fig. 3. DA levels started to increase already at the first sampling (20 min after the administration of 10 mg/kg of buspirone) although the significance and maximal effect (+90%) was reached only in the second sampling made 40 min after the drug injection. DA remained at this level during the 3 hours of observation. HVA and DOPAC concentration in the dialysed fluid was also measured. As shown in the graphs of Fig. 3 the output of these metabolites was also increased by buspirone.

DISCUSSION

Many pharmacological evidences show that buspirone cannot be considered a typical neuroleptic although it shares with these drugs some biochemical effects [5, 6, 12]. *In vitro* experiments indicate that buspirone is provided with affinity for the D_2 dopaminergic receptors [3, 5, 6, 12]. *In vivo* studies suggest that the drug acts on dopaminergic receptors in antagonistic ways [3, 5, 8]. In fact, busprione, similar to neuroleptics which enhance DA release, elevates DOPAC and HVA in both striatum and nucleus accumbens, two brain areas with a high concentration of dopaminergic nerve terminals. This effect has been confirmed by the present results that were obtained in both *ex vivo* and *in vivo* experiments. However elevation of the acid metabolites is not in itself a decisive evidence of increased neurotransmitter release. In fact the concentration of these metabolites may be elevated as a consequence of an increased intraneuronal metabolism determined by drugs acting on the storage vesicles such as reserpine or by drug inhibiting the nerve impulse flow such as gamma-butirrolactone (GBL). With regard to this aspect, the fact that, differently from other drugs which stimulate DA release [10], buspirone failed to increase the basal concentration of 3MT, a metabolite which is index of DA output, is rather intriguing. It is now generally accepted that DA collected by microdialysis tubes placed in specific brain areas represents the output of the neurotransmitter by the nerve terminals. Therefore the increase in DA observed in the dialysed fluid after buspirone is a direct evidence that this drug does indeed increase DA release. Furthermore, although 3MT basal levels were not increased, the accumulation of this extraneuronal metabolites after MAO inhibition, was clearly enhanced, which also suggests an increase neurotransmitter output. In fact this increased accumulation is not observed when the model is applied to situations of increased intraneuronal metabolism of DA as in the case of GBL administration ([11] and unpublished observations).

Buspirone is transformed in the organism into 1-PP. The fact that all the above described effects were absent when 1-PP was administered instead of buspirone, confirms that the dopaminergic effects are due to the drug itself. However the increase of DA release does not seem to affect DA receptors because chronic treatment with buspirone does not enhance their density differently from what observed after neuroleptics [3].

Our experiments in which *in vivo* pulse voltammetry was used, has confirmed that DA metabolism in nucleus accumbens is also affected [8]. The DOPAC increase in this area was identical to that observed in the striatum. This could indicate that the increase in DA metabolism is not due to a neuronal feedback which is absent in nucleus accumbens [2] but probably to blocking of the autoreceptors.

Buspirone effect in the accumbens which is known, together with other limbic structures, to be important in the emotional mechanisms, opens the possibility that some of the antianxiety property of this drug may be mediated by its action in this part of the brain. It is well documented that buspirone is active on noradrenergic as well as on serotonergic system. In particular the action of buspirone on the limbic area is not limited to the dopaminergic system: in fact a stimulating effect on serotonergic neurons probably due to its interaction with the $5HT_{1A}$ receptors, has been well documented ([6,9] and De Simoni, paper in preparation).

An aspect that remains to be elucidated is the consistent and significant decrease in DA concentration in the striatal tissue. This decrease is rather peculiar because no other neuroleptics have this effect. A working hypothesis that will be tested in the future is the possibility that buspirone may act on the DA storage vesicles.

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