Evidence that central 5-hydroxytryptaminergic neurones are involved in the anxiolytic activity of buspirone

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1 A two-compartment exploratory test was used to assess the role of central 5-hydroxytryptaminergic neurones in the anxiolytic activity of buspirone in rats.

2 Buspirone 0.1 mg kg^{-1} , administered subcutaneously 15 min before testing, significantly increased black-white transitions (BWT) in control rats but had no effect in animals injected intracerebroventricularly one week before with $150 \mu\text{g}$ 5,7-dihydroxytryptamine (in $20 \mu\text{l}$).

3 Infusion of buspirone in the median raphe (but not in the dorsal raphe) significantly enhanced BWT, at doses from $1 \mu\text{g}$ to $10 \mu\text{g}$ (in $0.5 \mu\text{l}$). Buspirone 5 and $10 \mu\text{g}$, but not $1 \mu\text{g}$, administered in the median raphe, significantly enhanced motor activity of rats during the first 10 min of testing in the activity cages.

4 The effect on BWT of $5 \mu\text{g}$ buspirone in the median raphe was completely antagonized in animals which had received either 5,7-dihydroxytryptamine intraventricularly, $150 \mu\text{g}$ (in $20 \mu\text{l}$), one week before or an infusion of $0.1 \mu\text{g}$ (in $0.5 \mu\text{l}$) (–)-propranolol in the same area 5 min before. (–)-Propranolol infused in the median raphe did not modify the effect of buspirone on locomotion.

5 Infusion of $5 \mu\text{g}$ buspirone (in $0.5 \mu\text{l}$) in the median raphe significantly enhanced punished responses in a conflict test with no effect on unpunished responding. Buspirone infused in the dorsal raphe had no effect on punished or unpunished responding over a wide dose range.

6 The results indicate that at the relatively low dose used in the present study buspirone produces an anxiolytic effect by acting on central 5-hydroxytryptaminergic neurones. It is likely that activation of 5-hydroxytryptamine_{1A}-receptors in the median raphe is involved.

Introduction

It has been suggested that the ability of buspirone, a non-benzodiazepine anxiolytic compound (Riblet *et al.*, 1982; Rickels *et al.*, 1982; Garattini *et al.*, 1982), to act as an agonist at central 5-hydroxytryptamine_{1A} (5-HT_{1A}) receptors is important for its anxiolytic activity (Peroutka, 1985; Eison *et al.*, 1986). The unavailability of selective antagonists at these receptors and the fact that the anxiolytic activity of buspirone is not identified in some models of anxiety (Pellow *et al.*, 1987; Critchley & Handley, 1987) have made it difficult to support this suggestion more directly.

Two studies recently examined the role of central 5-hydroxytryptaminergic neurones in the anxiolytic effect of buspirone by using intraventricular injections of 5,7-dihydroxytryptamine (5,7-DHT) or electrolytic lesions of the dorsal (DR) and median (MR)

raphe nuclei (Eison *et al.*, 1986; Davis *et al.*, 1988). Eison *et al.* (1986) showed that 5,7-DHT reduced the anticonflict effect of buspirone, whereas Davis *et al.* (1988) found that the ability of buspirone to block fear-potentiated startle was not modified in raphe lesioned rats. Apart from the differences in anxiety models and the means used to destroy central 5-HT-ergic neurones, a possible explanation of these findings is that pre- and postsynaptic 5-HT_{1A} receptors respectively are involved in the experiments of Eison *et al.* (1986) and Davis *et al.* (1988).

A difficulty with these studies is that both authors used 10 mg kg^{-1} buspirone, a dose causing profound changes in other transmitter systems such as catecholamines and acetylcholine (Kolasa *et al.*, 1982; Cimino *et al.*, 1983). In fact, even 1 mg kg^{-1} buspirone i.p. was shown, using *in vivo* voltametry, to increase striatal dopamine metabolism in rats (Loulot *et al.*, 1986). Doses of buspirone higher than

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1 mg kg⁻¹ may cause sedative effects (Merlo Pich & Samanin, 1986; Critchley & Handley, 1987) which further complicate the picture.

It has been recently found that the ED₅₀ of buspirone for inhibiting raphe 5-HT neuronal firing is 0.088 mg kg⁻¹ (Vander Maelen *et al.*, 1986). Interestingly, 0.1 mg kg⁻¹ buspirone was demonstrated to have anxiolytic activity in a two-compartment exploratory test in rats (Merlo Pich & Samanin, 1986). Even though apomorphine (0.2 mg kg⁻¹ s.c.) counteracted the effect of 0.1 mg kg⁻¹ buspirone s.c. and this dose of buspirone may enhance dopamine metabolism in the nucleus accumbens of freely moving rats (Louilot *et al.*, 1986), this does not exclude the possibility that the primary mechanism of action of buspirone at doses lower than 1 mg kg⁻¹ is on central 5-HTergic neurones.

The present study addressed this issue by examining the effects of 0.1 mg kg⁻¹ buspirone in the two-compartment exploratory test in rats injected intraventricularly (i.v.t.) with 5,7-DHT. Since in a previous study it was suggested that the MR may be specifically involved in the anxiolytic activity of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), another 5-HT_{1A} receptor agonist (Carli & Samanin, 1988), we administered various doses of buspirone into the MR or DR and studied its effect in a two-compartment exploratory test and in a conflict test previously shown to be sensitive to systemically administered buspirone (Merlo Pich & Samanin, 1986).

Methods

Male CD-COBS rats (Charles River, Italy), weighing 200–300 g were housed at constant room temperature (21 ± 1°C) and relative humidity (60%) with a regular light/dark schedule (light: 07 h 00 min–19 h 00 min). Food (Altromin pellets for rats) and water were freely available in the two-compartment exploratory test, whereas rats used in the test for conditioned suppression of drinking had limited access to water (45 min per day after testing).

Two-compartment exploratory test

The apparatus employed to test 'approach-avoidance behaviour' has been described previously (Merlo Pich & Samanin, 1986; Carli & Samanin, 1988). It consists of a conventional open field (100 × 100 cm), divided into two compartments: a square area (40 × 40 cm) in one corner of the open field with all surfaces blackened and a roof, fitted 35 cm from the floor, to prevent light entering from above (black compartment); the rest of the open field uniformly lit by a fluorescent lamp (white

compartment). Each rat was placed gently in the same peripheral lighted square close to the 'black' compartment to start the test session. The number of black-white transitions (BWT) between the two compartments was counted separately for 5 min by an observer sitting quietly at a distance of 2 m, unaware of the treatment. Whenever a rat entered a new square with all four legs an event was recorded. After testing each rat the floor was thoroughly cleaned. Testing was done between 14 h 00 min and 18 h 00 min. Animals were assigned randomly to different experimental groups.

Conflict test

The experimental chamber was a rectangular box (30 × 30 × 40 cm) with plastic sides and top and a metal grid floor. Through a hole in the middle of one wall, 4 cm in diameter, 8 cm above the grid floor, the rat had access to a stainless steel drinking tube, 7 mm in diameter, recessed 2 cm from the plane of the cage wall and connected to a plastic bottle containing approximately 100 ml of water. The support of the drinking tube contained a metal clip and leads connected to a shock device adjustable from 90 to 440 µA a.c. at 220 V. The grid floor served as the other pole to complete the shock circuit through the animal's paws. Thus only animals licking the tube received a shock under 'punished' condition. A tone signal was presented through a speaker mounted in the cage ceiling. The sensing device for the rat's contacts with the drinking tube was a photocell set horizontally in the tube support, its beam just clearing the end of the tube. The photocell beam was narrow (less than 2 mm) making it unlikely that the animal could break it without contacting the drinking tube. Direct observation indicated that in fact the beam was broken only when rats actually touched the tube. A digital programmer was used to control experimental contingencies and record responses. Three separate timers controlled cycles of tone, tone-plus-shock and silent period (no shock). Digital clocks recorded the total time of interruption of the photocell beam for each period.

During training, water-deprived rats were placed in the box without shock delivery, to locate the drinking tube and become accustomed to drinking from it. After drinking behaviour had stabilized (about 10 days of training), a schedule comprising cycles of 3 s (tone), 10 s (tone-plus-shock) and 25 s (silent period) was presented with the shock level set at 250 µA. Daily sessions lasted 10 min, yielding about 16 cycles of the sequence tone alone, tone-plus-shock and silent period. The animals were tested for drug effect when they had reached a fairly stable rate of responding during 10 min sessions (about 15 sessions). Each animal was assigned to

receive a series of counterbalanced single drug or saline injections into the MR or DR.

Motor activity

Motor activity was measured using eight wire grid cages (20.5 × 28 × 21 cm) each with two horizontal infrared photocell beams 3 cm above the floor along the long axis. Beam interruptions were recorded by an on-line microcomputer programmed in ONLI-BASIC (EuroCUBE System, Control Universal, U.K.). After 30 min habituation to the new environment, rats were injected with various doses of drug or saline and left in their home cage for 10 min. At the end of this period, they were placed in the activity cages and their motor activity recorded at 10 min intervals for 90 min.

Cannulae implantation, microinjection procedure and histological verification of guides

Rats were stereotaxically implanted under sodium pentobarbitone/chloral hydrate (equitensin) anaesthesia. One stainless steel guide cannula (23 gauge) was implanted to give access to the MR, AP +0.35, L 0.0 and V -2.6 or to the DR, AP +0.35, L 0.0 and V -0.6 (Konig & Klippel, 1963). In order to prevent clogging, 30 gauge stainless steel stylets were placed in the guide cannulae until the animals were given intracerebral injections.

On the day of the test the stylets were withdrawn and replaced by injection units (30 gauge stainless steel tubing) terminating 2 mm below the tip of the guides. Intracerebral injections of drugs or saline were made using a Harvard Apparatus compact infusion pump. Rats were hand-held while a 30 gauge injection needle was placed in the surgically implanted guide cannula. The injection needle was attached to the pump syringe by polyethylene tubing filled with drug or vehicle solution. Buspirone or (-)-propranolol HCl at the dose to be administered was dissolved in 0.5 µl saline and this volume was infused over a 1 min period. The injection cannula was withdrawn 30 s after the end of the infusion and the 30 gauge stylet replaced into the guide cannula.

Animals were returned to their home cages for 10 min before the test session commenced. Animals were tested only once in the two-compartment exploratory test and 4–5 times, each separated by two training sessions, in the conflict test.

In the experiments studying interactions between (-)-propranolol and buspirone, rats were infused with saline 0.5 µl or (-)-propranolol 0.1 µl in 0.5 µl into the MR. Five min later they were infused with buspirone 5 µg in 0.5 µl or saline 0.5 µl into the MR and left in their home cages 10 min before the test session began.

The locations of the cannulae were determined histologically after the experiments. Only data from rats in which the cannulae were located within the MR or DR nucleus were included in the results.

Injection of 5,7-dihydroxytryptamine

The rats, anaesthetized with equitensin, were immobilized in a Kopf stereotaxic instrument. 5,7-DHT creatinine sulphate 150 µg (calculated as free base) was dissolved in 20 µl of ascorbic acid solution (1 mg ml⁻¹) and administered into the right lateral ventricle. Control animals received only the ascorbic acid solution (vehicle). To protect noradrenaline containing neurones from the action of 5,7-DHT (Baumgarten *et al.*, 1973), 30 min before the injection of 5,7-DHT the rats were given desipramine (DMI) 25 mg kg⁻¹ i.p., an inhibitor of noradrenaline uptake into nerve endings (Samanin *et al.*, 1975).

In one experiment, animals were treated i.v.t. with 5,7-DHT or ascorbic acid solution and implanted with guide cannulae to give access to the MR. Animals treated with 5,7-DHT and their controls were caged singly until testing. After surgery, animals were allowed to recover for 7 days during which time they were accustomed to being handled.

At the end of the experiments rats treated with 5,7-DHT and their controls were killed by decapitation and their brains were rapidly removed for assay of 5-HT according to Achilli *et al.* (1985). When buspirone was infused in the MR, 5-HT was assayed in the forebrain and the remaining brainstem was used for histological examination of the cannulae locations.

Statistics

Data were analysed by one-way analysis of variance followed by Dunnett's *t* test. Two-way analysis of variance (ANOVA 2 × 2) (experiments with 5,7-DHT or (-)-propranolol) was followed by Tukey's test. Biochemical data were analysed by Student's *t* test. The motor activity data were processed by analysis of variance with split-plot design with factors 'time' within subjects and factor 'treatment' between subjects. In the experiment in which (-)-propranolol and buspirone were infused into the MR and activity measured in activity cages, each interval was analysed by ANOVA 2 × 2 followed by Tukey's test.

Drugs

5,7-DHT creatinine sulphate was purchased from SERVA, Feinbiochemical (Heidelberg F.R.G.). The various drugs were kindly provided by Bristol Myers (Evansville, Indiana, U.S.A.) (buspirone); ICI

Pharma. (Macclesfield, Cheshire, U.K.) ((-)-propranolol HCl); Ciba-Geigy (Origgio VA, Italy) (DMI) and Abbott (Saint-Remy-sur Avre, France) (sodium pentobarbitone). All drugs were dissolved in saline or distilled water.

Results

Two-compartment test

In agreement with previous findings (Merlo Pich & Samanin, 1986) in preliminary experiments buspirone showed anxiolytic activity in the two-compartment exploratory test only in a narrow dose range, 0.1 mg kg^{-1} being the most effective.

Figure 1 shows that buspirone 0.1 mg kg^{-1} s.c. administered 15 min before testing significantly increased BWT ($P < 0.01$, Tukey's test) in rats treated i.v.t. with vehicle but had no effect on BWT of 5,7-DHT-treated rats (ANOVA 2×2 ; Fint(1,33) = 11.2, $P < 0.01$). Rats receiving buspirone or saline

Figure 1 Effects of buspirone 0.1 mg kg^{-1} s.c. (Bus) or saline 2 ml kg^{-1} (Sal) on black-white transitions by vehicle (Sham) or 5,7-dihydroxytryptamine (5,7-DHT, $150 \mu\text{g}$ in $20 \mu\text{l}$) intraventricularly-treated rats. Buspirone was injected 15 min before the test session. ANOVA 2×2 , Fint(1,33) = 11.2 ($P < 0.01$), ** $P < 0.01$ vs Sham + saline (Tukey's test). †† $P < 0.01$ vs Sham + Bus (Tukey's test).

Table 1 Effects of buspirone infused into the rat median raphe nucleus on black-white transitions (BWT) and motor activity

Drug	(μg in $0.5 \mu\text{l}$)	BWT	Activity counts
Saline		1.4 ± 0.4	27.7 ± 15.8
Buspirone	1.0	$6.7 \pm 1.1^*$	42.8 ± 6.6
Buspirone	5.0	$16.0 \pm 2.5^{**}$	$69.3 \pm 9.4^{**}$
Buspirone	10.0	$18.0 \pm 2.0^{**}$	$71.5 \pm 13.4^{**}$

The values are means $\pm$ s.e.mean of 6 rats per group. Separate groups of animals were used.

BWT: * $P < 0.05$; ** $P < 0.01$ compared to saline; Dunnett's *t* test.

Activity counts: ** $P < 0.01$ compared to saline; Tukey's test.

had comparable 5-HT depletion in the brain. 5,7-DHT depleted brain 5-HT to about 20% of controls. Levels of 5-HT ($\pm$ s.e.mean) were (in ng g^{-1}): sham + saline 332.5 ± 11.9 ; sham + buspirone 303.5 ± 15.6 ; 5,7-DHT + saline $70.1 \pm 6.9^{**}$ and 5,7-DHT + buspirone $54.8 \pm 5.1^{**}$ (** $P < 0.01$ vs sham, Student's *t* test). Three animals injected with buspirone but which responded to 5,7-DHT treatment with only a slight decrease in brain 5-HT levels (less than 40%) showed a high number of BWT and were not included in the results.

Table 1 shows the effect of buspirone administered in the MR on BWT and activity counts. Control rats (saline) showed a clear preference for the dark area (very few BWT) but, when infused with buspirone into the MR, frequently moved towards the illuminated area, with a significant increase in BWT (ANOVA $F(3,20) = 21.0$, $P < 0.001$). The effect of buspirone infused into the MR on BWT was dose-dependent between $1 \mu\text{g}$ ($P < 0.05$, Dunnett's *t* test) and $5 \mu\text{g}$ ($P < 0.01$, Dunnett's *t* test). Buspirone $10 \mu\text{g}$ ($P < 0.01$, Dunnett's *t* test) did not raise the number of BWT further. Infusions of buspirone (1 and $5 \mu\text{g}$ in $0.5 \mu\text{l}$) into the DR did not increase BWT at any dose tested ($F(2,16) = 0.35$, NS) (data not shown).

Motor activity

To examine whether increased motor activity might account for the effect of buspirone on BWT, doses of 1.0, 5.0 and $10 \mu\text{g}$ buspirone were infused into the MR and motor activity was measured in the activity cages. As shown in Table 1, $5 \mu\text{g}$ and $10 \mu\text{g}$ but not $1 \mu\text{g}$ significantly enhanced motor activity (measured as increase in photocell interruptions) during the first 10 min of testing ($P < 0.01$, Tukey's test).

Figure 2 Effects of buspirone 5 μ g in 0.5 μ l (Bus) or saline 0.5 μ l (Sal) injected into the median raphe nucleus on black-white transitions by vehicle (Sham) or 5,7-dihydroxytryptamine (5,7-DHT, 150 μ g in 20 μ l) intraventricularly treated rats. Buspirone was injected 10 min before the test session. ANOVA 2 $\times$ 2, Fint(1,34) = 13.3 ($P < 0.001$). ** $P < 0.01$ vs Sham + saline (Tukey's test). †† $P < 0.01$ vs Sham + Bus (Tukey's test).

Effects of buspirone in animals pretreated with 5,7-DHT or (–)-propranolol

Figure 2 presents the effects on BWT of buspirone infused into the MR of rats which had received 5,7-DHT 150 μ g i.v.t. or vehicle 7 days before. 5,7-DHT did not modify BWT but completely antagonized the effect of buspirone 5 μ g in 0.5 μ l infused into the MR (ANOVA 2 $\times$ 2; Fint(1,34) = 13.3, $P < 0.001$). Fore-brain levels of 5-HT were markedly reduced in 5,7-DHT-treated animals. Levels of 5-HT ($\pm$ s.e.mean) were in ng g⁻¹: sham + saline 258 $\pm$ 9.4; sham + buspirone 258.8 $\pm$ 6.1; 5,7-DHT + saline 34.9 $\pm$ 2.3**; 5,7-DHT + buspirone 32.0 $\pm$ 2.4** (** $P < 0.01$ vs sham, Student's *t* test).

The effects on BWT of (–)-propranolol and buspirone infused into the MR, are shown in Figure 3a. A dose of 0.1 μ g (–)-propranolol in 0.5 μ l infused into the MR had no effect on BWT but completely antagonized the increase induced by 5 μ g (in 0.5 μ l) buspirone infused into the MR (ANOVA 2 $\times$ 2; Fint(1,19) = 10.32, $P < 0.01$). (–)-Propranolol 0.1 μ g (in 0.5 μ l) infused into the MR tended to enhance motor activity (Figure 3b), and had an additive effect

Figure 3 Effects of (–)-propranolol 0.1 μ g in 0.5 μ l (Prop), saline 0.5 μ l (Sal) and buspirone 5 μ g in 0.5 μ l (Bus) injected alone or in combination into the median raphe nucleus on black-white transition (a) and activity counts (b). (a) Black-white transitions ANOVA 2 $\times$ 2, Fint(1,19) = 10.3 ($P < 0.01$). * $P < 0.05$, ** $P < 0.01$ vs Sal + Sal (Tukey's test). † $P < 0.05$, †† $P < 0.01$ vs Sal + Bus (Tukey's test). (b) Activity counts ANOVA 2 $\times$ 2, Fint = 0.69 (NS).

with 5 μ g buspirone (ANOVA 2 $\times$ 2; Fint(1,24) = 0.69, NS; $P < 0.05$, Tukey's test).

Conflict test

Figure 4 shows the effects of buspirone infused into the MR on punished and unpunished responding in a conflict test. After about 20 days of training the animals showed low, stable levels of responding during shock presentation periods and a high level of responding during unpunished periods. Infusions of 5 μ g buspirone into the MR increased the drinking time during the punished period of the schedule to 204% of controls (ANOVA; F(2,16) = 20.5, $P < 0.01$) but had no effect on unpunished responding (ANOVA; F(2,16) = 1.15, NS). Buspirone 1 μ g administered into the MR had no effect on

Figure 4 Effects of buspirone (Bus, 1 and 5 µg in 0.5 µl) and saline injected into the nucleus raphe medianus on punished (■) and unpunished (□) responding in a conflict test. Control values (mean ± s.e.mean) for punished and unpunished responses were 1.8 ± 0.4 and 260 ± 19.1 (s), respectively. ** $P < 0.01$ compared to saline (Dunnett's t test).

either component. Infusions of buspirone into the DR did not release shock-suppressed drinking (ANOVA; $F(4,32) = 0.17$, NS) or modify unpunished responding (ANOVA; $F(3,32) = 0.18$, NS) over a wide dose range (0.1–10 µg) (data not shown).

Discussion

An intracerebroventricular injection of 5,7-DHT which depleted brain 5-HT by about 80% completely antagonized the increase of BWT caused by 0.1 mg kg^{-1} buspirone. This suggests that under the present experimental conditions buspirone acts on brain 5-HTergic neurones to exert its anxiolytic activity. This agrees with the results of Eison *et al.* (1986) who found that the anticonflict effect of 10 mg kg^{-1} buspirone was reduced in 5,7-DHT-treated rats. In a previous study we were unable to find an anticonflict effect, in a procedure using conditioned suppression of drinking, with doses of buspirone higher than 1.2 mg kg^{-1} . A possible explanation for these differences is that Eison *et al.* (1986) used a modified version of Vogel's test in which untrained rats were required to lick a drinking tube

during punishment with electric shocks. Since 8-OH-DPAT, another 5-HT_{1A} receptor agonist (Middlemiss & Fozard, 1983), was recently shown to enhance drinking in naïve but not in habituated rats (Carli & Samanin, 1988), effects of buspirone on primary drives may have influenced the punished responses in the Vogel test. Eating caused by 5-HT_{1A} agonists in sated rats has been found to be prevented by intraventricular injection of 5,7-DHT (Bendotti & Samanin, 1986).

As regards the failure of Davis *et al.* (1988) to antagonize the effect of 10 mg kg^{-1} buspirone on the fear-potentiated startle response with raphe lesions or 5-HT antagonists, it is possible that non-5-HT mechanisms are involved in the effect of this dose of buspirone in this particular model. Buspirone can have different effects on different populations of dopaminergic pathways (Louilot *et al.*, 1986) and the central nucleus of the amygdala has been shown to be critical for the potentiated startle response (Hitchcock & Davis, 1986). Therefore, as suggested by Davis *et al.* (1988), at a dose of 10 mg kg^{-1} some action of buspirone on dopamine receptors in this limbic area may be involved in its effect on the potentiated startle response.

As stated in the introduction, the dose of buspirone used in the present study is closely related to that inhibiting the firing of 5-HTergic neurones in raphe nuclei (Vander Maelen *et al.*, 1986) and in a previous study administration of 8-OH-DPAT into the nucleus raphe medianus (but not the dorsalis) enhanced BWT in the two-compartment exploratory test and punished responding in conditioned suppression of drinking (Carli & Samanin, 1988). It is likely therefore that the anxiolytic effect of buspirone found in the present study depends on its ability to activate 5-HT_{1A} receptors in midbrain raphe nuclei.

To prove this more directly, we administered various doses of buspirone in the MR or DR and studied its effect in the two-compartment exploratory test. At doses ranging from 1 to 10 µg buspirone enhanced BWT of rats when administered in the MR but not in the DR. Although 5 and 10 µg buspirone in the MR markedly enhanced motor activity in the activity cages, 1 µg had no significant effect, making it unlikely that the anxiolytic-like activity is due to increased general activity of the animals.

That an increase in activity does not necessarily influence the effect of buspirone on BWT is shown by the fact that administration of 0.1 µg (–)-propranolol into the MR completely antagonized the anxiolytic-like effect of buspirone without changing its effect on motor activity. (–)-Propranolol has affinity for 5-HT₁ receptors (Nahorski & Willcocks, 1983) and was demonstrated to block the inhibitory effects of 5-HT_{1A} agonists on the firing of 5-HTergic neurones in the raphe area (Sprouse & Aghajanian,

1986). Although a possible contribution of (–)-propranolol effects on β -adrenoceptors cannot be excluded with certainty, it is likely that 5 μ g buspirone in the MR produces an anxiolytic effect by acting on 5-HTergic neurones. Involvement of 5-HT in the effect of buspirone is further supported by the finding that this dose of buspirone in the MR had no effect in 5,7-DHT-treated animals.

To confirm that buspirone administered in the MR has anxiolytic activity, we studied its effect in a conflict test where punished and unpunished responses may provide information, respectively, on anxiolytic activity and effects on sensorimotor performance (Merlo Pich & Samanin, 1986; Carli & Samanin, 1988). Five μ g buspirone significantly enhanced punished responses while 1 μ g caused only an insignificant increase. No significant effects on unpunished responding were found with any dose. These results can be taken to indicate that the anxiolytic effect of buspirone is not secondary to changes in sensorimotor performance. Over a wide dose range, buspirone administered into the DR did not significantly modify punished or unpunished

responses. These findings confirm a previous study (Carli & Samanin, 1988) suggesting that activation of 5-HT_{1A} receptors, specifically in the MR, produces anxiolytic-like effects in rats.

In conclusion, in this study systemic administration of a relatively low dose of buspirone caused anxiolytic-like effects which require the integrity of central 5-HTergic neurones. Anxiolytic effects were also found when the drug was administered into the MR (but not the DR) and the effects were prevented by selective destruction of 5-HTergic neurones or administration of (–)-propranolol in the same brain region. Buspirone administered in the MR increased locomotion but this effect could be separated from the anxiolytic activity. It thus appears that activation of 5-HT_{1A} receptors in one brain region rich in 5-HT cells (the MR nucleus) causes anxiolytic activity. Since activation of these receptors results in reduced 5-HT transmission (Vander Maelen *et al.*, 1986), the findings are compatible with the hypothesis that 5-HT in the brain acts by favouring responses to signals of punishment and anxiety.

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