J. Pharm. Pharmacol. 1988, 40: 221-223
Communicated July 27, 1987

Dopamine-mediated behaviours produced in naive mice by bromocriptine plus SKF 38393

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Abstract—The ability of bromocriptine and SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine) to produce some dopamine-mediated behaviours has been assessed in mice. Soon after injection, SKF38393 produced moderate increases in grooming and sniffing which were not very intense, while bromocriptine (with or without SKF38393) inhibited all grooming behaviour. Bromocriptine alone also depressed rearing and sniffing in the first hour and SKF38393 alone was without effect on rearing, but the combination produced a marked increase in the incidence of both rearing and sniffing, both of which behaviours appeared to be stereotyped. When bromocriptine-induced locomotor stimulation was peaking about 3 h after injection, as measured in automated activity cages in previous studies, there was an increase in sniffing and rearing, the incidence of which was unaffected by the addition of SKF38393, perhaps due to the shorter duration of action of SKF38393 than of bromocriptine. The data indicate that the D-1 agonist SKF38393 can qualitatively and quantitatively alter the behavioural spectrum produced by the D-2 agonist bromocriptine.

We have recently shown that the selective dopamine (DA) D-1 receptor agonist SKF38393 can modulate the locomotor stimulation produced by the D-2 agonist bromocriptine the degree of modulation being dependent upon the degree of DA depletion (Jackson & Hashizume 1986, 1987). In mice with granular stores of DA depleted by reserpine, and DA synthesis inhibited by α-methyl-*p*-tyrosine, bromocriptine and SKF38393 administered separately produced no stimulation. The combination, however, produced a marked and coordinated increase in locomotor activity. In contrast, this interaction was more subtle in mice with DA stores and synthesis intact. Thus, the initial depressant response to bromocriptine was shortened by concomitant SKF38393 administration, which therefore resulted in a more rapid onset of the stimulant response, together with a more marked stimulant phase (Jackson & Hashizume 1987). Observation of the animals to which the combination had been administered indicated the presence of various behaviours, including forward locomotion, sniffing and some rearing. These individual behaviours were not assessed quantitatively and we now report an analysis of some additional behaviours produced by bromocriptine and SKF38393, alone and in combination, in naive mice.

Materials and methods

Male QS mice (20–30 g) were used. Immediately after drug injections, they were placed individually into ventilated Perspex boxes (95 mm wide, 73 mm high and 70 mm deep) and their behaviour assessed for 1 min every 30 min for 7 h, a time which spanned the full effect of the drugs used, by an observer blind to the drug treatment. We have found that the standard 1 to 5 scale of stereotypy as used, for example, in assessing stereotypy produced by apomorphine, is inappropriate for bromocriptine-induced stereotypy (Wallis & Jackson unpublished data), so we assessed the presence or absence of individual behaviours, including whether the animal was asleep, awake but still, grooming, sniffing or rearing. The animal was scored as grooming, sniffing or rearing if that behaviour was performed in a repetitive way over the 1 min observation period. In some cases, the behaviour produced was not intensively stereotyped, especially after SKF38393 by itself, also an animal might display two of the behaviours at the same time (e.g. sniffing and rearing) and then each behaviour was scored as being present (rearing and grooming are mutually exclusive behaviours). The most interesting data were the scores of grooming, rearing and sniffing, and these are illustrated in Fig. 1 as the percentage of animals (i.e. percentage prevalence) displaying each of the behaviours each 30 min. Thus, a 50% score indicates that 6 of the 12 animals displayed that particular behaviour at a given time. Data were compared statistically by χ^2 tests.

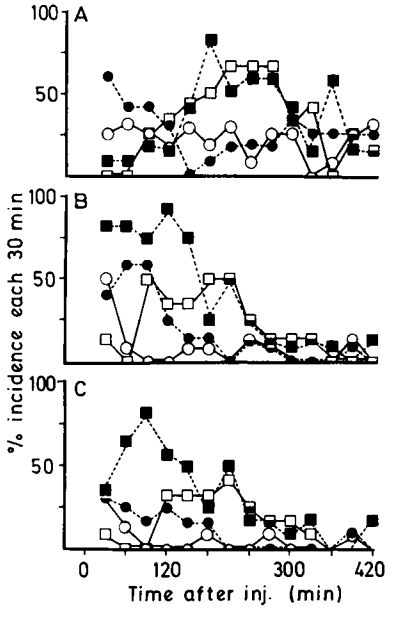

FIG. 1. Mice were injected with bromocriptine vehicle plus SKF38393 vehicle (○), bromocriptine vehicle plus SKF38393 (6 mg kg^{-1}) (●), bromocriptine (10 mg kg^{-1}) plus SKF38393 vehicle (□) or bromocriptine plus SKF38393 (10 and 6 mg kg^{-1}, respectively) (■) and placed into individual perspex cages. The incidence of grooming (panel A), sniffing (panel B) and rearing (panel C) was assessed each half hour. The data in these three panels represent the percentage of animals displaying each behaviour, with 12 mice being assessed in each challenge group.

The drugs and doses used were bromocriptine (Sandoz, Australia), 10 mg kg^{-1} i.p., dissolved in aqueous 0·01% tartaric acid and SKF38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine) (Research Biochemicals Inc. USA 6 mg kg^{-1} i.p., dissolved in aqueous 0·01% ascorbic acid). The injection volume was 10 mL kg^{-1}. These doses are the same as those used in our locomotor activity studies so that direct comparison can be made between the automatically measured

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activity and the behaviour as assessed here. Four groups each consisting of 12 mice were used: the first received the two vehicles; the second received the SKF38393 vehicle plus bromocriptine; the third received SKF38393 plus the vehicle for bromocriptine and the fourth received both drugs. Each mouse was used once only, and drug administration was scheduled to ensure that variation arising from handling, diurnal and biological sources was distributed equally across the four treatment groups.

Results

The results are illustrated in Fig. 1. At the first 30 min reading, SKF38393 produced a moderate increase in grooming behaviour ($P=0{\cdot}09$ compared with vehicle-challenged animals, panel A), although this was of a lower intensity than that seen in the two bromocriptine-challenged groups several hours after injection (see below), and appeared qualitatively like the grooming behaviour seen in the vehicle-challenged animals. In contrast, grooming was almost absent in mice challenged with bromocriptine (with or without SKF38393) for the first two readings. After the first hour, the incidence of grooming decreased in the group administered SKF38393. However, in the groups challenged with bromocriptine, there was a slow rise in grooming behaviour which peaked 150 to 270 min after injection, and which was unaffected by the presence of SKF38393. While the grooming behaviour induced by bromocriptine itself was not intense, it was not usually interrupted by rearing and sniffing. Although bromocriptine itself also depressed both rearing (panel C) and sniffing (panel B) in the first hour, the addition of SKF38393 radically altered this pattern, with the drug combination producing a marked increase in the incidence of both behaviours. In the case of sniffing, a significant difference between the two bromocriptine-challenged groups was evident at 30, 60 and 120 min ($P<0{\cdot}01$), and a trend at 90 min ($P=0{\cdot}2$) and 150 min ($P=0{\cdot}1$). In the case of rearing, the difference between the two bromocriptine-challenged groups was most marked at the 60 ($P<0{\cdot}001$) and 90 ($P<0{\cdot}001$) min readings, although a similar trend was evident at 30 ($P=0{\cdot}13$) and 120 ($P=0{\cdot}22$) min. In the second hour, there was an increase in sniffing, rearing and grooming in the group administered bromocriptine alone and these behaviours were maintained until at least the 300 min reading. Thus, by the 180 min reading, the two bromocriptine-challenged groups were displaying similar behaviours, perhaps as a consequence of the shorter duration of action of SKF38393 compared with bromocriptine (Jackson et al, unpublished data). Interestingly, SKF38393 itself produced a significant increase in the incidence of sniffing behaviour 60 and 90 min after injection, compared with animals challenged with the vehicles alone (both $P<0{\cdot}05$, panel B). This sniffing did not seem to be as intense as that seen in the animals challenged with bromocriptine plus SKF38393. Bromocriptine (10 mg kg^{-1}) induced locomotor stimulation as measured in automated photocell cages (Jackson & Hashizume 1987) is maximal between 120 and 300 min after injection. When SKF38393 was administered as well as the bromocriptine, the stimulation, while peaking around the same time, has a more rapid onset and sometimes a greater peak activity.

Discussion

As expected, and in agreement with Molloy & Waddington (1984, 1985), Arnt (1985) and Braun & Chase (1985), SKF38393 produced a moderate increase in grooming behaviour soon after injection. Interestingly, there was also an increase in the incidence of sniffing (but not of rearing) 60 and 90 min after injection, as assessed under the present conditions. This increase in grooming and sniffing was not evident at later times. Increased sniffing after SKF38393 challenge has also been reported by Molloy & Waddington (1985).

During the first hour, BRC by itself produced a marked decrease in rearing, sniffing and grooming behaviours, probably due to its potent DA autoreceptor agonist activity and resultant reduction in DA synthesis and release (Dolphin et al 1977; Yarbrough et al 1984). Thus, the depression of locomotor activity as measured in automated activity cages (see Jackson & Hashizume 1987) is accompanied by a general decrease in grooming, rearing and sniffing behaviours. However, when SKF38393 was given together with the bromocriptine, a complete change in behaviour emerged. This combination produced a marked increase in both rearing and sniffing, behaviours which were notably depressed by bromocriptine alone. The response of the animals to the combination of bromocriptine and SKF38393 in the present study is in general agreement with observations reported by Braun & Chase (1985) using SKF38393 and LY171555 (D-(-)-*trans*-(4a*R*)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline HCl), and with Mashurano & Waddington (1986) using SKF38393 and RU24213 (3-[2-[*N*(phenylethyl)-*N*-(propyl)amino]ethyl]phenol HCl). The latter authors also noted that the combination of D-1 and D-2 agonists produced more intense stereotypy than the D-2 agonist alone. An interaction between bromocriptine and SKF38393 has also been reported by Robertson & Robertson (1986) who, using the supersensitive rotating rat model, showed that the combination produced substantial rotation, while the individual drugs alone at the same doses were inactive. These authors also demonstrated a similar interaction between SKF38393 and LY171555. Thus, in its interaction with D-1 agonists, bromocriptine resembles other D-2 agonists. It is interesting that apomorphine, a non-selective D-1/D-2 agonist also produces intense stereotyped behaviour, including sniffing and rearing, but with little grooming. However, grooming can be elicited by apomorphine if the animals are treated with the D-2 antagonist metoclopramide (Molloy & Waddington 1985). Thus these findings, and the present results, suggest that sniffing and rearing as behaviours are maximally produced by concomitant D-1 and D-2 receptor stimulation, while grooming may involve a more significant D-1 component. It was of interest, therefore, that bromocriptine itself also produced an increase in grooming behaviour several hours after injection at a time corresponding to that when maximal locomotor stimulation occurs. The relation between this grooming, and that produced by SKF38393 in the first hour, is unknown, although the bromocriptine-induced grooming may well involve a D-1 component in the same way as the locomotor stimulation produced by BRC in naive mice (Jackson & Hashizume 1987).

In conclusion, the combination of bromocriptine and SKF38393 in naive mice produces a complex behavioural syndrome, which is characterized not only by an increase in coordinated forward locomotion (Jackson & Hashizume 1987), but by a marked change soon after injection in the incidence of rearing and sniffing. The increase in rearing and sniffing observed in the present study may partly explain our previous data in which we reported that SKF38393 when given with bromocriptine, shortened the initial depressant phase and thereby produced a more rapid onset of the stimulant phase (Jackson & Hashizume 1987).

This research was supported by The National Health and Medical Research Council (Australia) and by Astra Alab (Sweden). SBR is on leave of absence from the Research Laboratories of Astra Alab AB, Södertalje, Sweden. Sandoz (Australia) is thanked for generous donations of bromocriptine.

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J. Pharm. Pharmacol. 1988, 40: 223–224
Communicated July 23, 1987

Paper 014/7606/7/2

High doses of L-naloxone but neither D-naloxone nor β-funaltrexamine prevent hyperthermia-induced seizures in rat pups

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Abstract—The effects of the non-specific opiate antagonist L-naloxone and the inactive isomer D-naloxone, as well as the specific mu receptor antagonist β-funaltrexamine, have been examined on hyperthermia-induced seizures in unrestrained 15 days old rats. Saline-injected animals exposed to an ambient temperature of 40°C showed a gradual increase in body temperature reaching a maximum of 42 ± 0·1°C at 50 min exposure. At this time all the pups had seizures and died. Similar results were obtained when the animals were pretreated with different doses of D-naloxone and β-funaltrexamine. Rats pretreated with L-naloxone also showed an increase in rectal temperature; but the temperature was lower than in saline-injected animals. Only high doses of L-naloxone prevented seizures and deaths. These data indicate that endogenous opioid peptides may play a role in seizures induced by hyperthermia and that receptors other than mu receptors could be involved in hyperthermia-induced seizures.

There is increasing evidence that endogenous opioid peptides may play a role in the pathophysiology of seizures, although pro- and anti-convulsant effects of opiates and endogenous opioid peptides have been reported (Frenk 1983; Tortella et al 1985). Both pro- and anti-convulsant effects could be explained by activation of different populations of opioid receptors. The existence of at least three distinct receptor types, mu, kappa and delta, has been suggested from in-vitro and in-vivo experiments, but that a particular response is mediated by a single subtype of opioid receptors has been difficult to establish, the major obstacle being the lack of selective opioid antagonists. Naloxone does not distinguish adequately between the different receptor types (Sawynok et al 1979). Recently, it has been suggested that the non-equilibrium antagonist β-funaltrexamine is selective for mu receptors (Ward et al 1982).

We have investigated the role of endogenous opioid peptides and opioid receptors, particularly the mu receptor (of which β-funaltrexamine is a selective antagonist) on seizures induced by hyperthermia in immature rats.

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Methods

Wistar albino rat pups aged 15 days and 34 ± 2·4 g were used. Acute hyperthermia was induced by placing rats in a ventilated incubator (UNITEMP) at a constant temperature of 40°C with 55% humidity. Rats were unrestrained during the experiments. Rectal temperatures were measured with a thermometer (ELLAB TF 3) connected to RM 6 thermocouple probes. The temperatures were recorded immediately after placing the rats in the chamber (time 0) and thereafter at 10 min intervals for a total of 90 min. The rats were injected intraperitoneally with 0·2 mL of 0·9% NaCl (saline), D-naloxone (1 or 10 mg kg^{-1}) and L-naloxone (1 or 10 mg kg^{-1}) at time 0; β-funaltrexamine was administered subcutaneously at a dose of 1·2 or 5 mg kg^{-1} 30 min before the experiments. The behavioural changes during exposure to hyperthermia were observed. The animals became hyperactive with scrambling movements in apparently random directions. After 30 min, generalized seizure activity appeared and this was followed by death.

Drugs used were: naloxone hydrochloride (Endo Laboratories Inc. New York, NY), β-funaltrexamine hydrochloride (Research Biochemical. Inc. Wayland, MA).

Data were analysed by Student's *t*-test and analysis of variance.

Results

Table 1 shows the results obtained with the different experimental groups. Saline-injected rats had a progressive increase in temperature reaching a maximal value of 42 ± 0·1°C at a 50 min exposure. Both groups injected with L-naloxone also showed a marked increase in body temperature, however, temperatures were significantly lower than in saline-injected animals ($P < 0·001$). The groups treated with D-naloxone and β-funaltrexamine did not show significant differences compared with the control group.

At 30 min, saline-injected animals had a temperature of 40·8 ± 0·2°C and 13% showed generalized tonic-clonic seizures (Table 1). In this group the rectal temperature was 42 ± 0·1°C after 50 min exposure and 100% of the animals had seizures.

The rats treated with L-naloxone 1 mg kg^{-1} (data not represented) had a rectal temperature of 40·9 ± 0·5°C at 50 min,