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BRIEF COMMUNICATION

Changes in Motor Activities Induced by Microinjections of the Selective Dopamine Agonists LY 171555, Quinpirole Hydrochloride, and SK&F 38393 Into the Habenula Nucleus

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THORNTON, E W , J A C EVANS AND A WICKENS *Changes in motor activities induced by microinjections of the selective dopamine agonists LY 171555, quinpirole hydrochloride, and SK&F 38393 into the habenula nucleus* PHARMACOL BIOCHEM BEHAV 26(3) 643–646, 1987 —The effects on behaviour of microinjections into the habenula complex of selective agonists for dopamine D-1 (SK&F 38393) and D-2 (LY 171555) receptors were documented in a holeboard, open-field test The D-2 agonist reduced grooming responses, locomotor activity and rearing behaviour In contrast, the D-1 agonist increased rearing and locomotor activity but was without effect on grooming responses Neither drug produced significant effects on inspective hole exploration. The data extend findings of behavioural consequences of central D-1 receptor activation and provide direct evidence in support of the functional and behavioural importance of intrahabenular dopamine receptor sites. The findings are consistent with suggestions for feedback regulation of habenular efferents to midbrain dopaminergic neurons. Effects of both receptor agonists on some responses but not others indicates potential complex interactions between D-1 and D-2 receptors within the habenula

Habenula Dopamine LY 171555 SK&F 38393 Motor activity Holeboard test ,

THE habenula has been suggested to be a pivotal nucleus through which influences of limbic forebrain may be relayed to those midbrain nuclei which are the source of ascending monoamine systems [18,21]. As such it represents a potentially important "upstream" nucleus for regulation of monoamine activity including dopaminergic systems through efferents to the ventral tegmental area (VTA) and substantia nigra (SN) [2,8]. Support for this notion is found in the attenuated turnover of dopamine during stress in frontal cortex following lesions of the habenula [9]. More directly, inhibitory effects on the activity of dopamine neurons have been demonstrated following electrical stimulation of the habenula [2]. The nature of this inhibition has been confirmed and further elaborated in pharmacological investigations [13]. From the functional perspective, the behavioural response during stress following lesions of the habenula is characterised by a response inflexibility [19] which is similar to that induced by a decrease in central dopamine function [3]. Such lesions also attenuate the behavioural effects of the dopaminergic drug nomifensine in a stressful swim test [20].

Anatomical evidence has shown that the habenula itself receives dopamine efferents with projections from the interfasicular nucleus to the medial habenula and, more extensively, from the medial VTA paranigral area to the caudal two-thirds of the medial portion of the lateral habenula nucleus [15]. This finding would suggest that there may be feedback regulation of efferent habenular activity to the dopamine cells of the midbrain. Certainly, systemic administration of the dopamine agonist apomorphine alters the metabolic activity of the habenula [10] although this may have been effected through indirect effects to the habenula from dopaminergic rich forebrain areas from which non-dopaminergic afferents arise [18].

In the present study we provide more direct evidence for intrahabenular dopamine function by examining the effects on behaviour of microinjection of dopamine agonists into the

TABLE 1

BEHAVIOURS OBSERVED IN THE HOLEBOARD OVER A 20 MINUTE TEST PERIOD FOLLOWING INTRAHABENULAR INJECTIONS OF EITHER LY 171555 OR SK&F 38393

Dose	Locomotion	No Rears	Time Rearing	No Grooms	Time Grooms	No Dips	Time Dips
			LY 17155 Data				
0 (vehicle)	91 3	18 2	47 7	15 9	228 8	12 1	24 0
	±6 2	±2 3	±6 6	±1 6	±27 1	±1 5	±3 4
6 μg	67 3	14 0	33 8	10 9	229 7	10 5	26 5
	±5 9	±1 9	±5 0	±1 2	±29 7	±1 7	±6 4
12 μg	60 1*	10 9	28 6	5 9*	89 4*	7 6	33 3
	±5 4	±1 9	±5 3	±0 8	±16 8	±1 3	±7 2
24 μg	73 5	23 5	57 0	7 8	177 6	8 4	19 4
	±5 6	±2 7	±7 0	±1 0	±25 6	±1 2	±3 3
			SK&F 38393 Data				
0 (vehicle)	66 5	18 8	48 1	11 4	331 7	9 6	29 0
	±11 3	±3 5	±11 1	±1 6	±47 3	±2 6	±11 3
1 25 μg	71 2	17 1	42 6	9 0	320 0	9 3	23 1
	±4 1	±2 4	±8 2	±1 8	±48 6	±1 8	±5 8
2 5 μg	81 5	24 3	73 5	10 0	310 0	8 7	19 9
	±8 3	±2 9	±11 4	±2 2	±49 8	±1 3	±3 8
5 0 μg	97 0†	27 9‡	77 0‡	11 3	306 9	11 6	29 2
	±11 5	±4 7	±14 8	±2 2	±51 4	±2 3	±8 2

Data represent the group Means (±SD) of the total incidence or duration (seconds) of response categories over the test period

Significant decrease relative to control (Tukey HSD), *=$p<0.01$

Significant increase relative to control, †=$p<0.05$, ‡=$p<0.01$

habenula. Different dopamine receptor sites have been implicated in different patterns of behaviour [4, 11, 12] Consequently, to provide more extensive information on such differentiation and on the habenula, we chose to examine effects of agonists with considerable specificity for the D-1 (SK&F 38393) and D-2 (LY 171555, quinpirole hydrochloride) receptor sites [17]

METHOD

Animals and Surgical Procedures

Twenty male Lister hooded rats (327±12 g), adapted to handling, were housed singly in a room maintained at 20–23°C with food and water freely available. Animals were anaesthetised with sodium pentobarbital (50 mg/kg) with a supplementary injection of 0.3 ml atropine sulfate given to reduce respiratory distress. Stainless steel guide cannulae with an outer diameter of 0.7 mm (Plastic Products, Roanoke, VA) were cut to 6.2 mm, implanted at an angle of 20° using coordinates taken from the Pellegrino and Cushman atlas [14]: A–P 3.8; L ±0.4; DV +0 7 from stereotaxic zero, and fixed to the skull with acrylic dental cement and screws Stainless steel stylets protruding beyond the guide cannulae were used to maintain the effectiveness of the cannulae between injections. Injection procedures commenced three days after surgery with ten animals assigned for injection with the D-1 agonist and the remainder, counterbalanced for weight, receiving the D-2 agonist.

Drugs and Injection Procedures

SK&F 39393 in the form of the hydrochloride (1-phenyl-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine, Smith, Kline and French, Philadelphia) was dissolved in 0.1% sodium metabisulphite to yield doses of 1.25 μg, 2 5 μg and 5.0 μg in a volume of 0.3 μl. The D-2 agonist LY 171555 (quinpirole hydrochloride Eli Lilly, Indianapolis) was dissolved in distilled water to produce doses of 6 μg, 12 μg and 24 μg in 0 3 μl Following three daily habituation sessions to the test apparatus and injection procedures, animals received one of each of the three doses of the appropriate drug or relevant vehicle on each of four test sessions spaced at three day intervals The order of drug dose administration was counterbalanced across animals Drugs were freshly prepared each day and, with animals held in the hand, injections were given bilaterally through injection cannulae cut 2 mm longer than the guide cannulae over a period of one minute, with cannulae left in situ for a further minute prior to removal

Behavioural Testing

On completion of injection animals were tested in a holeboard enclosure to provide measures of locomotor activity and directed exploration [5] The square enclosure with walls of 80 cm and 80 cm high was divided into nine equal squares with holes 4 cm diameter in each corner square below which were varied objects. The enclosure was illuminated with a 60 V lamp suspended 140 cm above the centre of

the floor. Each animal was placed singly in the holeboard for a 20 minute trial which was recorded on video-tape to be scored later without knowledge of treatment condition. At the end of each trial fecal boluses were removed and the apparatus wiped clean. General activity was related to the number of squares entered and rearing. Hole exploration was scored for both frequency and duration of head dipping with scores related to a criterion of both eyes going below the hole. The incidence and duration of grooming was also noted.

On completion of behavioural tests each animal received bilateral injections of cresyl violet dye and later was sacrificed, the brain removed and stored in sucrose formalin solution prior to subsequent histological examination to determine the site of injections

RESULTS

Examination of brain sections confirmed the integrity of the habenula and revealed injection sites to be accurately placed in medial and posterior parts of the medial and lateral habenular nuclei with no intrusion into adjacent nuclei in all but one animal In that brain, the tip was too dorsal with non-invasion into the habenula on both sides Data were therefore compiled from 10 rats with SK&F 38393 injections and only 9 rats receiving LY 171555. For both drugs the exact location of cannulae placements was found not to correlate with the extent of behavioural effects.

The effects of both drugs on behaviour components are summarised in Table 1 No signs of abnormal postures or motor responses such as stereotypies were evident in any animal following drug treatments

ANOVA statistical analyses were undertaken for each measure for both drugs with data arranged in two minute blocks over the 20 minute test period. All significant dose effects were further analysed using the Tukey HSD test All measures other than locomotor activity (squares crossed) presented a skewed distribution and a level of significance of $p<0$ 01 was adopted for rejection of the null hypothesis in these instances However, results reaching the 5% level of significance are given to indicate trends in the data

The ANOVA revealed a significant dose effect, $F(3,24)=3$ 3, $p<0$ 05, indicating a reduction in locomotion following LY 171555 injections, with further analysis confirming a significant effect at a dose of 12 μg relative to vehicle ($p<0$ 05)) There were additional ANOVA dose effects for LY 171555 on both the incidence ($F=9$ 6, $p<0$ 01) and frequency ($F=6$ 9, $p<0.01$) of grooming with again a maximal reduction at the middle dose ($p<0.01$). The tendency to change rearing did not quite reach the required level of significance ($F=4$ 1, $p<0$ 05)

In contrast to data for the D-2 agonist, the D-1 agonist SK&F 38393 showed significant dose effects on both the frequency, $F(3,27)=4$ 7, $p<0$ 01, and duration ($F=5$ 9, $p<0.01$) of rearing activity with subsequent analyses confirming significant increases for both measures at the highest dose relative to vehicle, $p<0.01$). There was also a dose effect on locomotor activity ($F=3$ 3, $p<0$ 05) with a significant increase in activity at the 5 μg dose ($p<0.05$)

For both drugs there were no significant effects of dose on components of hole exploration, while significant time effects for all measures was consistent with the steady decline of these measures over each test session The ANOVA's revealed no significant interactions between time and each behaviour component recorded for either drug.

DISCUSSION

Both the D-1 agonist SK&F 38393 and the D-2 agonist LY 171555 produced significant behavioural effects on motoric components of open-field behaviour recorded in the holeboard apparatus following local injection into the habenula. The primary effects on locomotor activity were in opposite directions, with D-1 receptor activity increasing locomotor activity and rearing whilst the D-2 activity reduced locomotor activity with a tendency to reduce rearing, at least at the lower doses LY 171555 produced significant reductions in grooming activity with SK&F 38393, in contrast, being without effect on this response measure. The differential direction of effects on activity and rearing for the two drugs testifies to the viability of the cannula preparation and precludes interpretation of these results as a consequence of physical damage to the habenula from the repeated injection procedures

The comparison of behavioral effects of the two drugs may be confounded by the doses selected and the paucity of data on the relative potencies of the drugs following central injections. The doses chosen are, however, representative of those used in other studies of direct central application [7,16], and, whilst a wide dose range was possible with the D-2 agonist, the highest dose of SK&F 38393 was determined by the limited solubility for the small injection volume.

Behavioural effects resulting from systemic administration of dopamine or dopamine agonists are believed to be primarily mediated by D-2 receptors and the significance of D-1 receptors questioned despite increasing evidence for behavioural effects [6, 11, 12, 17] The present data clearly confirm the importance of both D-1 and D-2 receptors in the habenula for motor activity and possibly rearing responses. These results extend, therefore, the limited data available on effects of central injection of specific dopamine receptor agonists and add to the growing evidence for the functional importance of D-1 receptors, including the similar finding of increased activity following injection of SK&F 38393 into the nucleus accumbens [6] In contrast to the results following systemic injections where D-1 agonists are active in producing increases in grooming [12], these results suggest a D-2 receptor contribution within the habenula for grooming behaviour

It is not possible from these results to specify whether the changes in behaviour reported are affected through direct feedback control of efferent habenular activity to dopamine cells of the VTA as the anatomical and physiological evidence might suggest. However, the data are not inconsistent with such a notion since changes in motor activity following manipulation of the mesolimbic dopamine pathway have been well documented Nevertheless, the differential effects of D-1 and D-2 agonists on activity means that such regulation may well be complex and may even involve interactions between the two receptor sites as previously suggested for other dopamine terminal sites [1,17].

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