Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 985-995, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00234-7

Dopamine D_1 and D_2 Receptor Ligands Modulate the Behaviour of Mice in the Elevated Plus-Maze

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Received 24 April 1994

RODGERS, R. J., E. M. NIKULINA AND J. C. COLE. *Dopamine D_1 and D_2 receptor ligands modulate the behaviour of mice in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **49**(4) 985-995, 1994. — To further our understanding of the potential role of dopamine in mechanisms of anxiety, the effects of four dopamine receptor ligands were examined in an ethological version of the murine elevated plus-maze test. The D_1 receptor partial agonist, SKF 38393 (2.5-20.0 mg/kg), had minimal behavioural activity in this test, whereas the selective D_1 receptor antagonist, SCH 23390 (0.025-0.2 mg/kg), had dose-dependent but behaviourally nonspecific effects. Quinpirole (0.0625-0.5 mg/kg), a D_2 receptor agonist, had no effects at low doses but severely disrupted locomotion and exploration at the highest doses tested. In marked contrast to the lack of effect or nonspecific effects seen with the other ligands tested, the D_2 receptor antagonist, sulpiride (2.5-20.0 mg/kg), produced an unambiguous anxiolytic-like profile under present test conditions. Although none of the doses tested adversely affected general activity, clear antianxiety effects were observed on both traditional and novel (i.e., risk assessment) behavioural measures. Data are discussed in relation to the relative importance of D_1 and D_2 receptor mechanisms in plus-maze anxiety, and the need to further assess D_2 involvement through the use of more selective compounds.

Elevated plus-maze Ethological analysis Risk assessment Dopamine SKF 38393 SCH-23390 Quinpirole Sulpiride

TRADITIONALLY, neurochemical and pharmacological research on the neurobiology of anxiety has focused on the roles of GABAergic, serotonergic, and noradrenergic systems [for reviews: (28,54)]. However, several lines of evidence combine to suggest that dopaminergic (DA) mechanisms may also have a modulatory role in emotional behaviour. For example, in vitro and in vivo methods have shown that acute exposure to stress (such as foot shock, restraint, social defeat) activates mesolimbic/cortical DA systems and that such effects can be attenuated by antianxiety drugs (5,13,15,27,34,43,45,47). Evidence also suggests that mesolimbic DA mechanisms may be involved in susceptibility to stress-induced gastric ulceration (21), and in the behavioural profiles of nervous pointer dogs (22). Studies in clinical and preclinical pharmacology provide support for these neurochemical findings. Although at least five DA receptor subtypes are now recognized [for review: (20)], the initial identification of D_1 and D_2 receptors (30) provided the major stimulus for research aimed at defining particular functional roles for DA receptor subtypes. In this context, early clinical data showed that small doses of the DA receptor antagonists, chlorpromazine (nonselective), and haloperidol (D_2), can be as effective as benzodiazepines against anxiety [for review: (48)]. These findings have more recently been replicated with the mesolimbic-selective D_2 receptor antagonist, sulpiride (59,63).

Anxiolytic-like effects have also been reported for DA receptor antagonists in a variety of animal models. Thus, in rats, low doses of trifluoperazine have antianxiety effects in a traditional conlfict paradigm (11), while haloperidol and sulpiride produce anxiolytic-like profiles in punished drinking and light/dark exploration tests (40). Although further research has confirmed the anxiolytic potential of the D_2 antagonist, sulpiride, data on haloperidol have been confounded by the compound's potent motoric effects. More specifically, sulpiride and tiapride have been shown to exert anxiolytic effects in the murine light-dark exploration test (12), whereas

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sulpiride, but not the D_1 receptor antagonist SCH 23390, reduces hyperdefensiveness in previously defeated male mice (41). In contrast, even low doses of haloperidol have been found to produce nonspecific effects in the mouse light–dark test (12). This problem does not appear to reflect a species difference in that similar, behaviourally disruptive effects of haloperidol have been observed in the elevated plus-maze test in both rats (31,39) and mice (9). The finding that SCH 23390 reduces emergence latencies into a brightly lit white compartment (56) is, to our knowledge, the only direct evidence for anxiolytic activity of selective D_1 receptor antagonists.

Research with DA receptor agonists provides further support the involvement of the D_2 receptor subtype in anxiety. Low doses of apomorphine have been reported to have anxiolytic efficacy in clinical populations (17), and to produce haloperidol-reversible reductions in defensive ambivalence/flight in murine resident-intruder tests (14). In a detailed series of studies, Hjorth and colleagues (23,24) observed biphasic effects with apomorphine and both enantiomers of 3-PPP (D_2 agonists) in the Vogel conflict procedure; low doses were found to produce anxiolytic-like effects that were attributed to an action at presynaptic autoreceptors, while high doses exerted anxiogenic-like effects thought to be mediated via postsynaptic sites. In this context, the dopamine releaser, dexamphetamine, and the novel D_2 agonist, RU 24926, have also recently been reported to have anxiogenic-like actions in a modified mouse light–dark exploration test (56). Similarly, quinpirole (D_2 agonist), but not SKF 38393 (D_1 agonist), has been shown to induce hyperdefensiveness in male mice confronted with a nonaggressive opponent (2,6,41,44). A similar finding has more recently been reported for another D_2 agonist, Ro 41-9067 (3). Quinpirole has also been found to increase anxiety in murine social interaction and light–dark exploration tests (19). Despite this apparent consistency in the effects of DA receptor agonists, a number of contradictory findings have been reported. Thus, SKF 38393 has been found to have weak anxiogenic-like effects in a modified light–dark exploration test (56) but to have no effect in the rat elevated plus-maze test (29). Furthermore, despite its anxiogenic-like effects in other tests, quinpirole has been reported to be without effect (2,29), or even to have anxiolytic-like effects (2), in the rat elevated plus-maze test.

The aim of the present study was to examine in detail the effects of D_1 and D_2 receptor agonists and antagonists on the behaviour of mice in the elevated plus-maze test of anxiety. This animal model of anxiety has been validated for both rats and mice (33,39) and is sensitive to both increases and decreases in anxiety (51). Recent work from this laboratory has confirmed the advantages of an ethological approach to the scoring of behaviour in the plus-maze. This form of the test records not only the traditional indices of anxiety (open-arm entries and time) but also a range of specific defensive behaviours displayed by mice on the maze. Risk assessment behaviours, including head-dipping, stretched attend postures, and closed-arm returns (see the Method section) have recently been shown to be very sensitive to anxiolytic and anxiogenic treatments. More specifically, these behaviours are consistently reduced by traditional and novel antianxiety drugs (9,10,52,53), and enhanced by anxiogenic drugs (52) and a variety of naturalistic stressors (49,50).

The compounds selected for study were the D_1 receptor partial agonist, SKF 38393 (55), the D_1 receptor antagonist, SCH 23390 (26), the D_2 receptor agonist, quinpirole (18), and the D_2 receptor antagonist, sulpiride (36). Although quinpirole also has affinity for D_3 and D_4 receptors (32,58), it was specifically chosen in preference to more selective D_2 receptor agonists because of its already well-established effects on defensive behaviour.

GENERAL METHOD

Animals

Subjects were 12–15-week-old adult male DBA/2 mice (Biomedical Services, University of Leeds), housed in groups of 10 (cage size: 45 × 28 × 13 cm) for at least 4 weeks prior to testing. They were maintained under a 12-h reversed light cycle (lights off: 0700 h) in a temperature-controlled environment (21 ± 1°C). Food and drinking water were freely available with the exception of the brief test periods. All mice were experimentally naive.

Drugs

Drugs used were quinpirole hydrochloride (formerly LY 171555; trans-(−)-4aR,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H(or 2H)-pyrazolo-(3,4,-g) quinoline monohydrochloride), (±)sulpiride ((±)-5-(Aminosulfonyl)-*N*-[(1-ethyl-2pyrrolidinyl)methyl]-2-methoxybenzamide), (±)SKF 38393 hydrochloride(2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine HCl), and R(+)SCH-23390 hydrochloride [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine HCl]. All compounds were purchased from Research Biochemicals Incorporated, USA, prepared freshly on test days, and administered IP (10 ml/kg) 20 min prior to testing. Quinpirole and SCH-23390 were dissolved in distilled water, SKF 38393 in saline, and sulpiride in acidified (1 drop 0.01 N HCl; pH = 6.3) distilled water.

Apparatus

The elevated plus-maze was a modification of that validated for NIH mice by Lister (33). Two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm) extended from a central platform (5 × 5 cm) making the shape of a plus-sign (like arms opposite), and the entire apparatus was raised to height of 45 cm above floor level. The maze floor was made from black Plexiglas while the side- and end-walls of the enclosed arms were made from clear Plexiglas. As previously reported [e.g., (9,10)], grip on the open arms was facilitated by inclusion of a raised edge (0.25 cm) and open-arm activity was further encouraged by testing under dim red light (3 × 60 W).

Procedure

All testing was conducted during the middark phase of the LD cycle (i.e., 1000–1600 h). To facilitate habituation, animals were transported from holding rooms to the laboratory at least 1 h prior to testing. For each study, mice were randomly allocated to treatment conditions (n = 10) and tested in a counterbalanced order. Testing commenced by placing an animal onto the central platform of the maze facing an open arm. A 5-min test duration was employed and, between subjects, the maze was thoroughly cleaned with damp and dry cloths. All test sessions were recorded by an overhead videocamera that was linked to a monitor and VCR in an adjacent room. Tapes were subsequently scored by an observer blind to treatment condition.

Behavioural Analysis

Videotapes were scored for both standard and novel behavioural measures. Standard measures were: number of open-

and closed-arm entries (arm entry defined as all four paws into an arm), total rears, and time spent on different sections of the maze (including the central platform). The spatial and temporal distribution of behaviour on the maze was additionally calculated as percent total for both frequency (percent open entries) and duration (percent time spent on open, centre, and closed sections) data.

A range of specific behaviours related to the defensive repertoire of the mouse were also recorded. These ethological measures comprised nonexploratory behaviour (the combined duration of immobility and grooming) and a cluster of risk assessment behaviours that characterize the profile of more cautious subjects: head dipping (dips; exploratory scanning over the sides of the maze); stretched-attend postures (SAP; forward elongation of the head and shoulders followed by retraction to original position), and closed-arm returns (exiting a closed arm with forepaws only and then returning into the same arm). Both head dipping and stretched-attend postures were differentiated by location as protected (i.e., occurring from the relative safety of a closed-arm or central platform) or unprotected (i.e., occurring on or from an open arm). Analogous to calculations for open entries/open time, data for head dipping and stretched-attend postures are presented both as totals and as percent protected values (%pDips, %pSAP; = protected/total × 100).

Statistical Analysis

Data were analyzed by single factor (drug treatment) or two-factor (drug treatment; maze location; repeated measures on location) analyses of variance (ANOVA). Where indicated (by significant/near significant F-values), further comparisons were performed using appropriate error variance terms from the ANOVA summary tables.

RESULTS

Experiment 1: SKF 38393

Data are summarized in Figs. 1-3 (left panels). ANOVA showed that, over the dose range tested, SKF 38393 had minimal behavioural effects in the murine elevated plus-maze. Nonexploratory behaviour was the only measure to be significantly altered by drug treatment, $F(4, 45) = 3.2$, $p < 0.025$; follow-up tests indicated that this effect was due to an increase

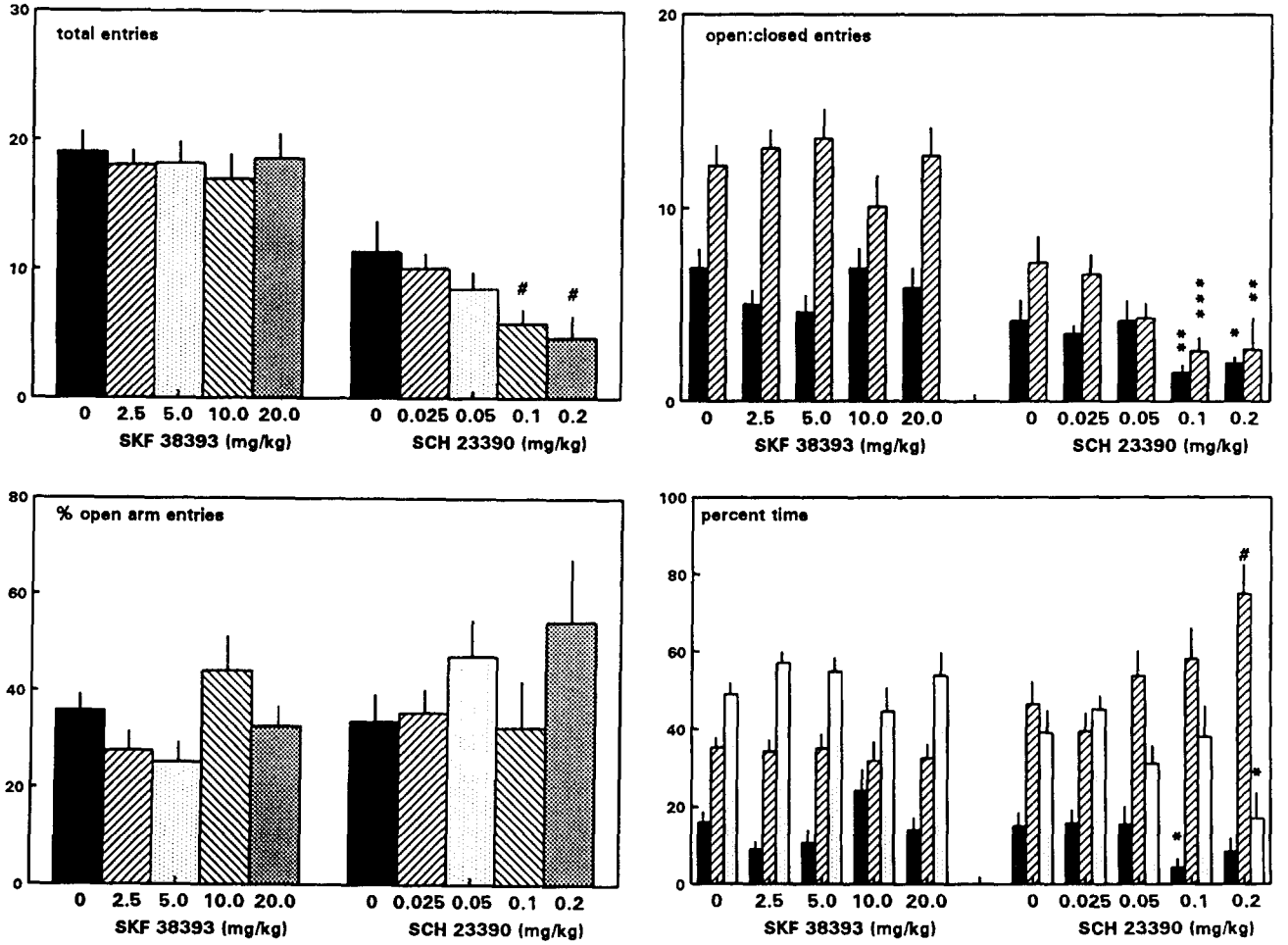

FIG. 1. Effects of SKF 38393 (2.5-20.0 mg/kg) and SCH-23390 (0.025-0.2 mg/kg) on total arm entries, open : closed entries, percent open-arm entries, and percent time spent on different maze sections in male mice tested in the elevated plus-maze. For open : closed entries chart, black bars = open arms; hatched bars = closed arms. For percent time chart, black bars = open, hatched bars = center platform, stippled bars = closed. Data are presented as mean values ± SEM. $*p < 0.05$, $**p < 0.025$, $***p < 0.01$, $\#p < 0.005$ vs. control.

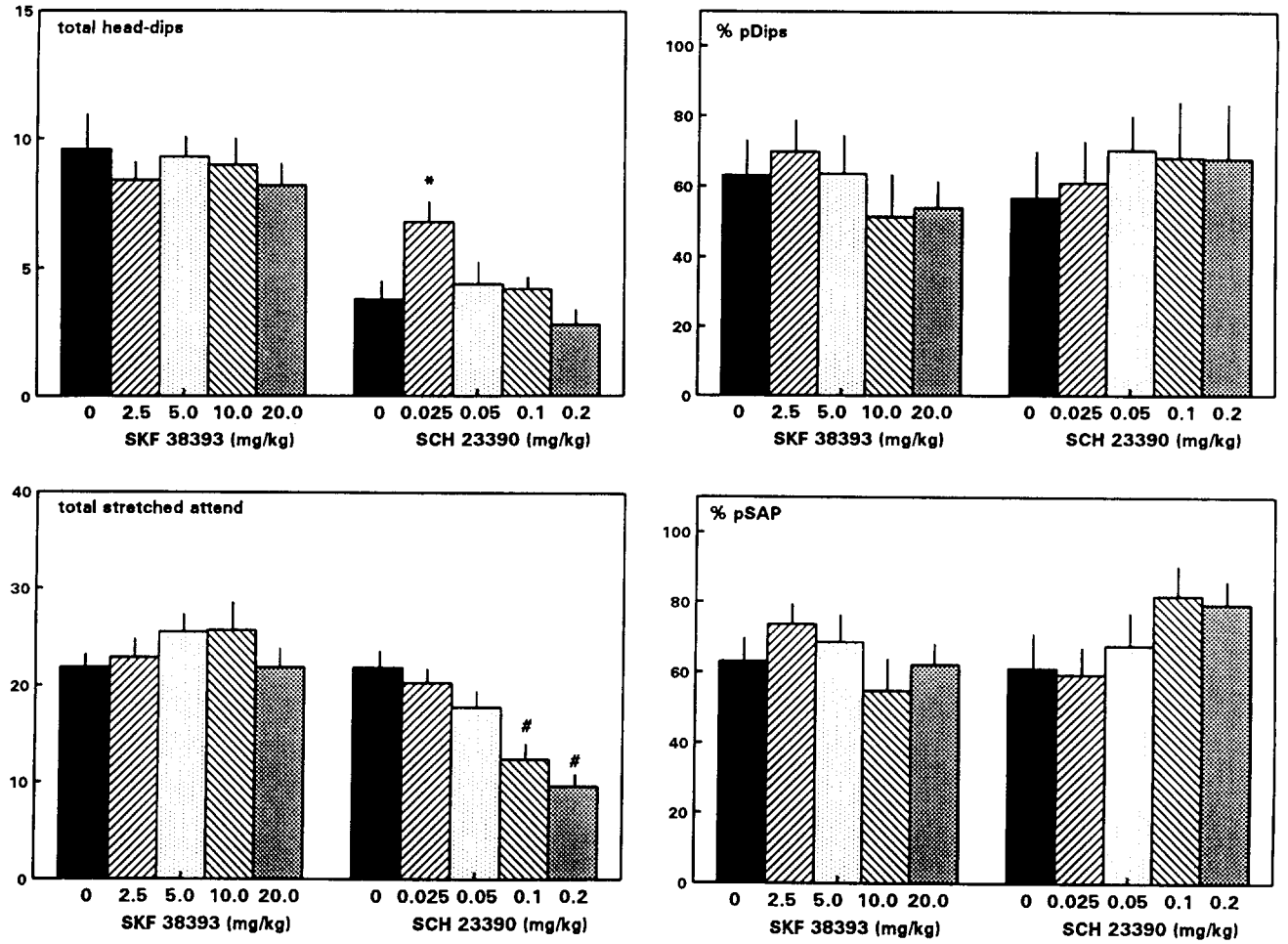

FIG. 2. Effects of SKF 38393 (2.5–20.0 mg/kg) and SCH-23390 (0.025–0.2 mg/kg) on total head dips, percent protected head dips (%pDips), total stretched-attend postures, and percent protected stretched-attend postures (%pSAP) in male mice tested in the elevated plus-maze. Data are presented as mean values ± SEM. *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$, #$p < 0.005$ vs. control.

observed at 20.0 mg/kg ($p < 0.01$). Although significant F-values were obtained for percent open-arm entries, $F(4,45) = 2.85$, $p < 0.05$, and percent open-arm time, $F(4, 45) = 2.99$, $p < 0.05$, further comparisons failed to reveal any differences between treatment and control conditions.

Other measures were completely unaffected by SKF 38393 (numbers in parentheses are F-values with $df = 4,45$ and $F_{crit0.05} = 2.61$): total entries (0.23), open entries (1.65), closed entries (1.02), rears (1.69), percent closed time (1.28), percent centre time (0.20), total dips (0.38), %pDips (0.76), total SAP (1.00), %pSAP (1.29), and closed-arm returns (0.32). Furthermore, although analysis of time spent on different maze sections revealed a clear preference of closed > centre > open, $F(2, 90) = 77.97$, $p < 0.001$, SKF 38393 did not alter this profile, $F(8, 90) = 1.44$, NS.

Experiment 2: SCH-23390

Data are summarized in Figs. 1-3 (right panels). ANOVA indicated significant effects of drug treatment on total entries, $F(4, 45) = 4.93$, $p < 0.01$, open entries, $F(4, 45) = 3.16$, $p < 0.025$, closed entries, $F(4, 45) = 3.77$, $p < 0.025$, and total rears, $F(4, 45) = 2.83$, $p < 0.05$. Further analyses showed that SCH-23390 reduced all arm entry measures at 0.1–0.2 mg/kg ($p < 0.05$ to $p < 0.005$), but inhibited rearing only at the top dose tested ($p < 0.05$).

Although percent open entries were not affected by drug treatment, $F(4, 45) = 1.29$, NS, significant changes were observed in time spent on different sections of the maze: percent open time, $F(4, 45) = 2.61$, $p < 0.05$; percent centre time, $F(4, 45) = 4.97$, $p < 0.01$; percent closed time, $F(4, 45) = 3.43$, $p < 0.025$. Follow-up tests showed that SCH 23390 significantly reduced percent open time at 0.1 mg/kg ($p < 0.05$) and percent closed-arm time at 0.2 mg/kg ($p < 0.05$), effects that were associated with a dose-related increase in centre time that reached significance at 0.2 mg/kg ($p < 0.005$). Further analyses confirmed this effect of SCH-23390: subjects generally had a clear preference for different sections of the maze, $F(2, 90) = 55.5$, $p < 0.001$, a pattern that was significantly altered by SCH-23390, $F(8, 90) = 4.30$, $p < 0.01$. Thus, control and 0.025 mg/kg groups did not differentiate between closed-arms and centre platform but preferred each of these areas to the open arms. In contrast, mice treated with intermediate doses (0.05–0.1 mg/kg) showed a rank order preference

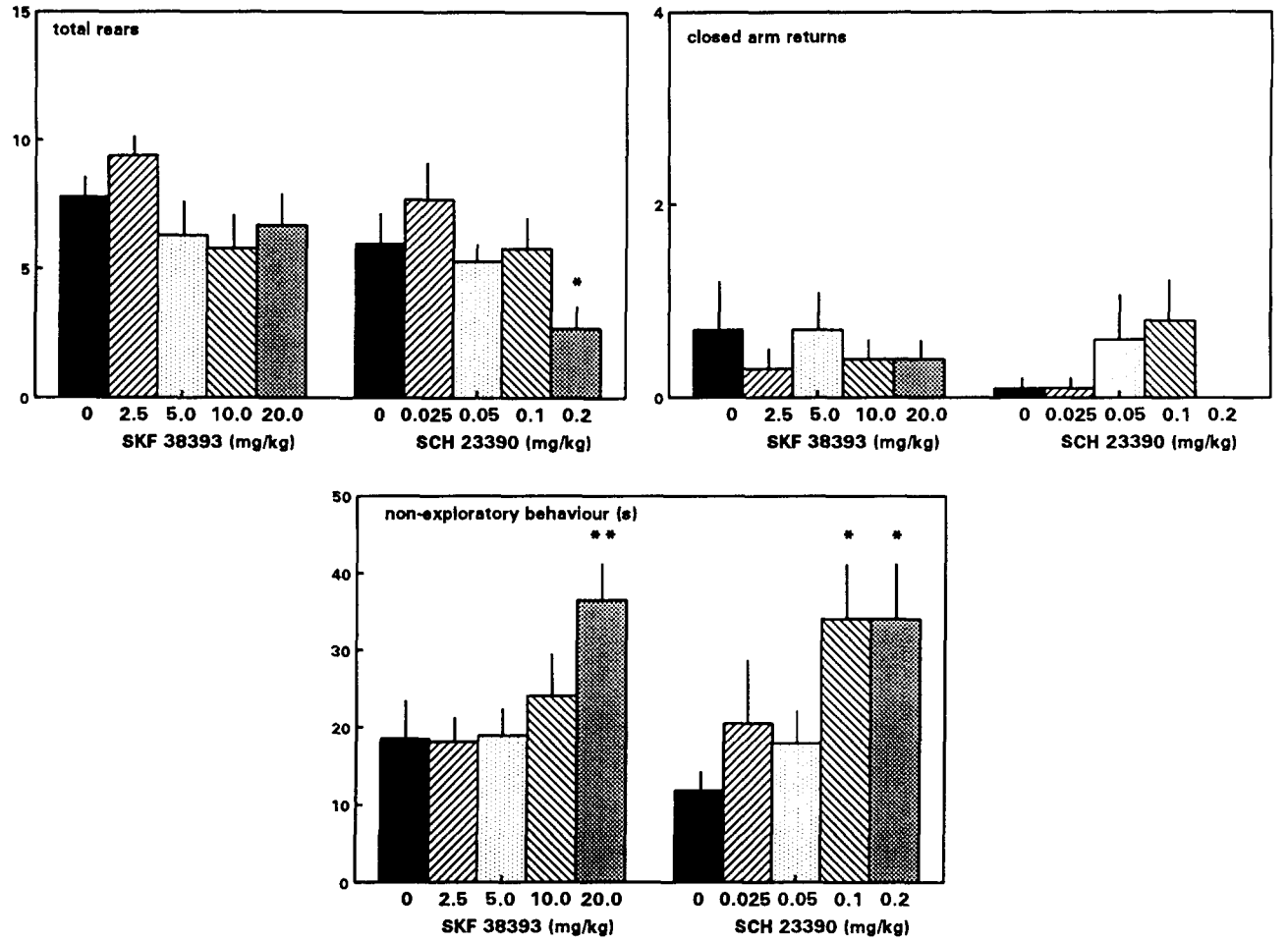

FIG. 3. Effects of SKF 38393 (2.5–20.0 mg/kg) and SCH-23390 (0.025–0.2 mg/kg) on total rears, closed-arm returns, and nonexploratory behaviour in male mice tested in the elevated plus-maze. Data are presented as mean values ± SEM. $*p < 0.05$, $**p < 0.025$, $***p < 0.01$, $\#p < 0.005$ vs. control.

of centre > closed > open, although those treated with the highest dose (0.2 mg/kg) displayed a profile of centre > closed = open.

Analysis of the new ethological measures revealed significant effects of SCH-23390 on total head dips, $F(4, 45) = 5.09$, $p < 0.01$, and total SAP, $F(4, 45) = 12.99$, $p < 0.001$, while the F-value for nonexploratory behaviour approached significance, $F(4, 45) = 2.37$ ($F_{crit0.05} = 2.61$). Further analyses showed that head dipping was increased at 0.025 mg/kg ($p < 0.005$) and nonexploratory behaviour at 0.1–0.2 mg/kg ($p < 0.05$), while SAP were reduced at 0.1–0.2 mg/kg ($p < 0.005$). SCH-23390 did not have a significant effect on closed-arm returns, $F(4, 45) = 1.51$, NS, %pDips, $F(4, 45) = 0.24$, NS, or %pSAP, $F(4, 45) = 1.79$, NS.

Experiment 3: Quinpirole

Data are summarized in Figs. 4–6 (left panels). ANOVA showed significant effects of quinpirole on total entries, $F(4, 45) = 14.22$, $p < 0.001$, open entries, $F(4, 45) = 5.12$, $p < 0.01$, closed entries, $F(4, 45) = 10.63$, and rearing, $F(4, 45) = 8.14$, $p < 0.01$. Follow-up tests indicated significant reductions in total entries, closed entries, and rears at 0.25–0.5 mg/kg ($p < 0.01$ to $p < 0.005$). Open entries were significantly reduced at the highest dose only ($p < 0.005$). The F-value for percent open entries approached significance, $F(4, 45) = 2.19$, NS ($F_{crit0.05} = 2.61$) with further comparisons indicating a significant increase in this measure at 0.5 mg/kg ($p < 0.025$).

Analysis of percent time data showed that quinpirole was without significant effect on percent open-arm time, $F(4, 45) = 0.20$, NS, percent centre time, $F(4, 45) = 1.52$, NS, or percent closed-arm time, $F(4, 45) = 1.72$, NS. Overall, mice displayed a clear preference for maze sections, $F(2, 90) = 27.36$, $p < 0.001$, a pattern that was apparently unaffected by drug treatment, $F(8, 90) = 1.41$, NS. However, it is interesting to note that although most groups did not discriminate the closed-arms and centre platform (but preferred both these areas to the open arms), mice treated with the highest dose of quinpirole showed a preference for the centre platform over both arm types.

Ethological measures were also affected by quinpirole treatment, with significant effects noted for head dipping, $F(4, 45) = 13.95$, $p < 0.001$, total SAP, $F(4, 45) = 14.11$,

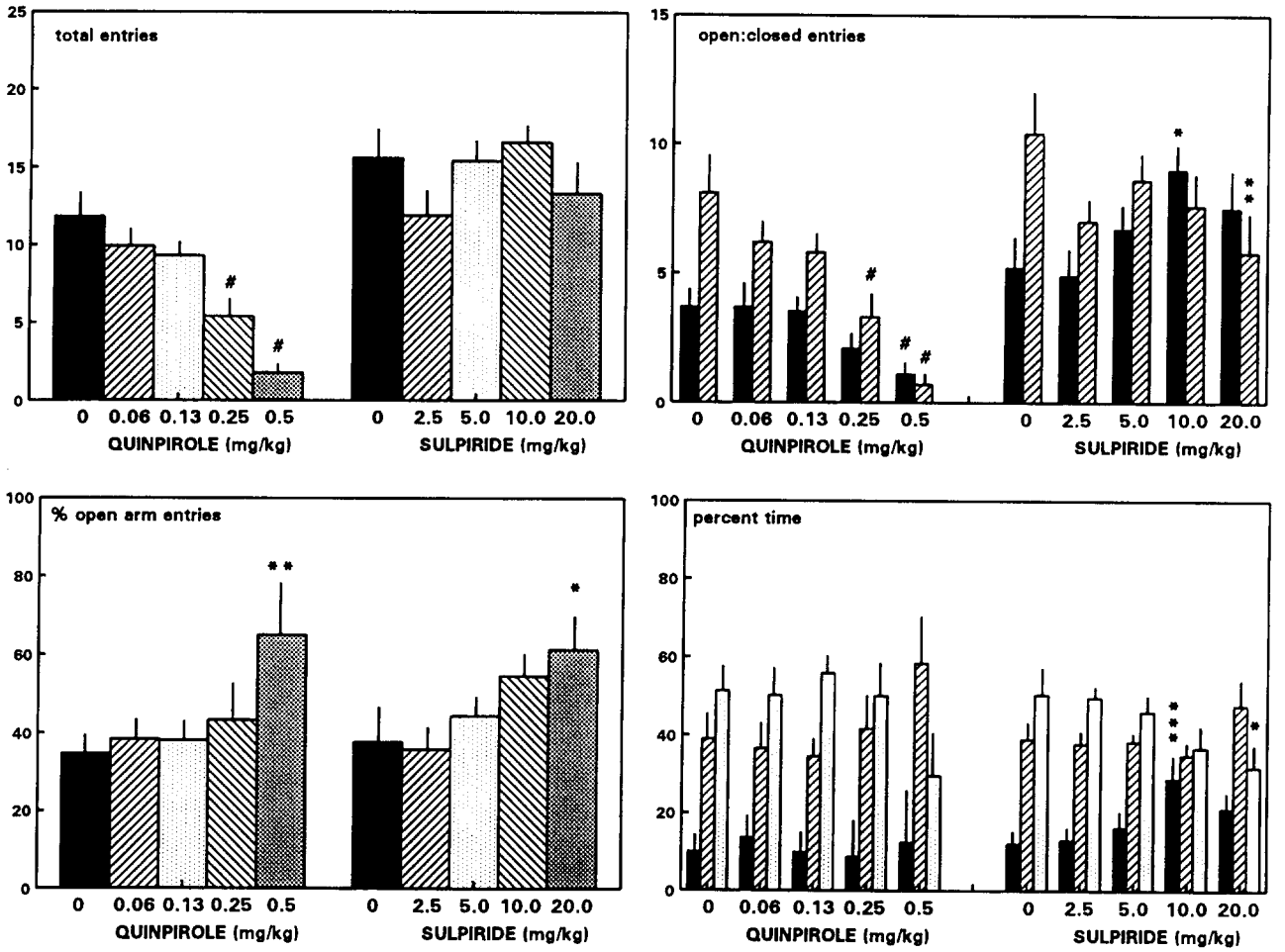

FIG. 4. Effects of quinpirole (0.0625–0.5 mg/kg) and sulpiride (2.5–20.0 mg/kg) on total arm entries, open : closed entries, percent open entries, and percent time spent on different maze sections in male mice tested in the elevated plus-maze. For open : closed entries, black bars = open arms, hatched bars = closed arms. For percent time chart: black bars = open, hatched bars = center platform, stippled bars = closed. Data are presented as mean values ± SEM. *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$, #$p < 0.005$ vs. control.

$p < 0.001$, and nonexploratory behaviour, $F(4, 45) = 22.30$, $p < 0.001$. Further comparisons showed that total head dipping and SAP were suppressed across the dose range tested ($p < 0.025$ to $p < 0.005$), while nonexploratory behaviour was markedly enhanced at 0.25–0.50 mg/kg ($p < 0.005$). Quinpirole did not significantly alter %pDips, $F(4, 45) = 0.88$, NS, %pSAP, $F(4, 45) = 2.43$, NS, or closed-arm returns, $F(4, 45) = 2.34$, NS.

Experiment 4: (±)Sulpiride

Data are summarized in Figs. 4–6 (right panels). ANOVA showed that sulpiride was without effect on total arm entries, $F(4, 45) = 1.43$, NS, closed entries, $F(4, 45) = 1.88$, NS, and rearing, $F(4, 45) = 0.26$, NS. The F-value for open-arm entries approached significance, $F(4, 45) = 2.56$, NS ($F_{crit0.05} = 2.61$), and follow-up tests revealed an increase on this measure at 10.0 mg/kg ($p < 0.05$). Consistent with this result, sulpiride produced a dose-dependent increase in percent open-arm entries, $F(4, 45) = 2.61$, $p < 0.05$, an effect that reached significance at 20.0 mg/kg ($p < 0.05$).

Analysis of percent time data indicated a significant treatment effect on percent open-arm time, $F(4, 45) = 3.35$, $p < 0.025$, an effect on closed-arm time that approached significance, $F(4, 45) = 2.43$, NS, and no effect on centre platform time, $F(4, 45) = 1.38$, NS. Further analysis confirmed a significant increase in percent open time at 10.0 mg/kg and a reduction in percent closed time at 20.0 mg/kg. Mice displayed a clear preference for different sections of the maze, $F(2, 90) = 30.2$, $p < 0.001$, a profile that was significantly altered by sulpiride, $F(8, 90) = 2.36$, $p < 0.05$. Control animals and those treated with 2.5 mg/kg sulpiride showed a rank order preference of closed > centre > open. Higher doses of sulpiride changed this pattern to closed = centre > open (5.0 mg/kg), no preference (10.0 mg/kg) and centre > closed = open (20.0 mg/kg).

Sulpiride treatment also produced effects on total head dipping, $F(4, 45) = 2.28$, NS, and %pDips, $F(4, 45) = 2.30$, NS, that approached significance. Further tests showed that total dips were enhanced at 10.0 mg/kg ($p < 0.05$) while %pDips were dose-dependently reduced by sulpiride, an effect reaching significance at 20.0 mg/kg ($p < 0.05$). Although total SAP were unaffected by treatment, $F(4, 45) = 1.35$, NS, a significant reduction %pSAP was observed, $F(4, 45) = 5.69$,

$p < 0.01$. The latter effect was apparent with all doses except 2.5 mg/kg ($p < 0.005$). No significant effects of sulpiride were noted for closed-arm returns, $F(4, 45) = 0.94$, NS, or nonexploratory behaviour, $F(4, 45) = 0.72$, NS.

DISCUSSION

In the elevated plus-maze test, changes in anxiety state are normally inferred from alterations in the proportion of open-arm entries (relative to total entries) and the proportion of time spent on the open arms (relative to test duration). Thus, in the absence of gross changes in locomotor activity, anxiolytics increase open-arm measures while anxiogenics have the opposite effect [for review: (51)]. Recent research has confirmed that the utility of this test can be further enhanced by recording specific behavioural acts and postures displayed by animals on the maze. A cluster of plus-maze behaviours, collectively referred to as risk assessment (4), has been identified as particularly sensitive to anxiety-related manipulations. These behaviours, which comprise stretched-attend postures, head dipping, and closed-arm returns, are reduced by traditional (9) and novel (9,10,53) anxiolytics, and are enhanced both by anxiogenic drugs (52) and by social/nonsocial stressors (49,50). The present study has utilized this more ethological approach to the plus-maze to characterize the effects of a range of dopaminergic agents on anxiety-related behaviour in mice. From a methodological viewpoint, it should perhaps be noted that, consistent with their nocturnal nature, we used animals maintained on a reversed light cycle and tested during the dark phase. It is, therefore, possible that, where any discrepancies arise between our data and those from other laboratories, light cycle may be a significant factor.

Our results show that the dopamine D_1 receptor partial agonist, SKF 38393 (2.5–10.0 mg/kg) did not alter any of the variables recorded, while the only effect observed at the highest dose tested (20 mg/kg) was an increase in nonexploratory behaviour. These negative findings are consistent with the lack of influence of this compound in the rat plus-maze (29) and murine defensive reactivity (41) tests, but are at variance with an apparent anxiogenic action in a modified mouse light–dark exploration test (56). However, the latter finding should be viewed cautiously as it is based purely on a drug-induced increase in emergence latency into a bright arena. The observation that SKF 38393 did not alter measures of general activity and exploration in the maze (i.e., total entries, rears, total head dipping) agrees well with reports that it has no effect on

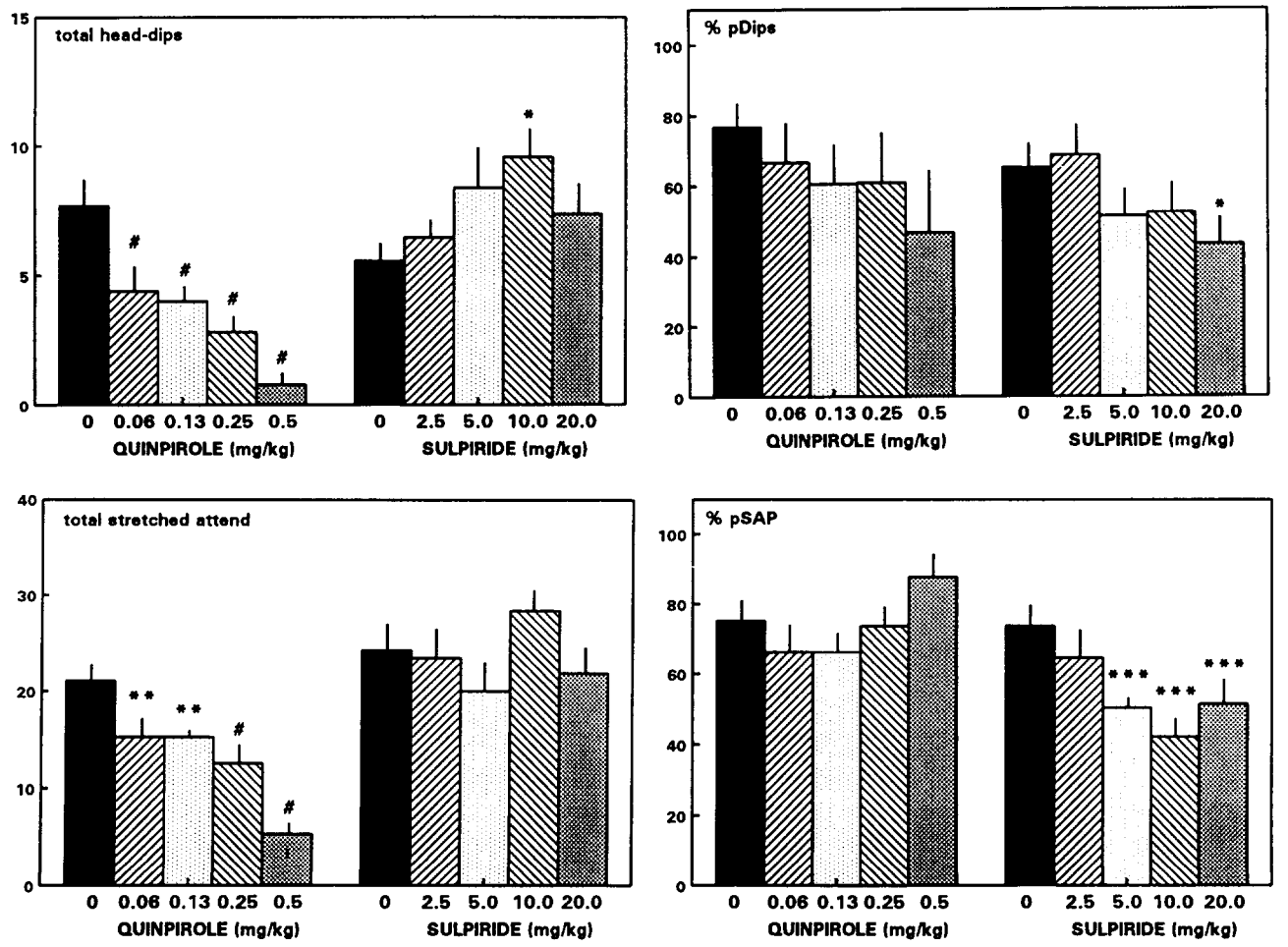

FIG. 5. Effects of quinpirole (0.0625–0.5 mg/kg) and sulpiride (2.5–20.0 mg/kg) on total head dips, percent protected head dips (%pDips), total stretched-attend postures, and percent protected stretched attend postures (%pSAP) in male mice tested in the elevated plus-maze. Data are presented as mean values ± SEM. *$p < 0.05$, **$p < 0.025$, ***$p < 0.01$, #$p < 0.005$ vs. control.

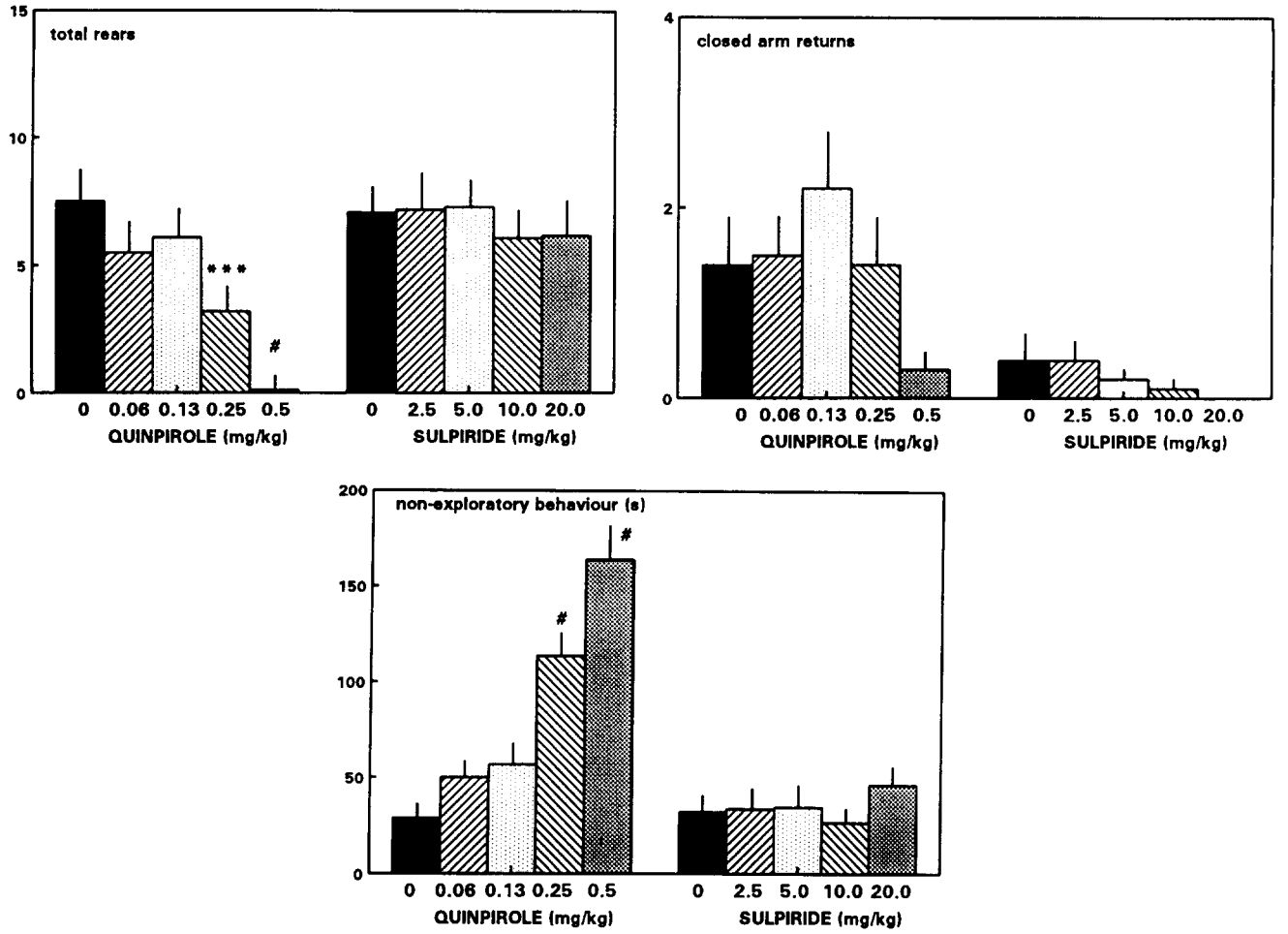

FIG. 6. Effects of quinpirole (0.0625–0.2 mg/kg) and sulpiride (2.5–20.0 mg/kg) on total rears, closed-arm returns, and nonexploratory behaviour in male mice tested in the elevated plus-maze. Data are presented as mean values ± SEM. $*p < 0.05$, $**p < 0.025$, $***p < 0.01$, $\#p < 0.005$ vs. control.

locomotor activity in this species up to 100 mg/kg (3,61). However, reductions in locomotion and rearing have been described in rats over a similar dose range (37), while both increases and decreases in activity have been seen with this D_1 receptor agonist in mice (57,62). As species, mouse strain and dose range cannot accommodate these discrepancies, they can only arise from differences in the test conditions employed (e.g., solitary vs. social context; habituated vs. nonhabituated animals). It may be pertinent to note that dopamine D_1 agonists, including SKF 38393, have been reported to increase grooming in rats (1,8). Although the currently observed increase in nonexploratory behaviour may reflect a similar enhancement of grooming in mice, the vertical camera angle precluded clear distinctions between such behaviour and immobility.

The apparent lack of involvement of D_1 receptor mechanisms in plus-maze anxiety is further supported by present findings with the selective antagonist, SCH-23390. Although little previous work has been done with this compound in anxiety-related tests, it has been reported to produce anxiolytic-like reductions in emergence into a brightly lit chamber (56), but to have no effect on hyperdefensiveness in previously defeated male mice (41). Our data show that SCH-23390 produces a dose-dependent behavioural suppression which, on most measures, reaches significance at 0.1–0.2 mg/kg. At these doses, total entries, total stretched-attend postures, and total rears were reduced, while nonexploratory behaviour was increased. These changes coincided with a dose-dependent increase in the proportion of time spent on the centre platform of the maze which, at 0.2 mg/kg, amounted to 75%. This profile is rather similar to that seen with haloperidol in the same test (10) and is consistent with the treated mice not moving very much from their initial starting position on the maze. The failure of SCH-23390 to alter other anxiety measures (e.g., percent open entries, %pDips, %pSAP, or closed-arm returns) suggests that observed reduction in percent open-arm time was artefactually related to general behavioural suppression observed at this dose. The conclusion that SCH-23390 exerts a nonspecific, behaviourally disruptive effect the the plus-maze test is consistent with many reports attesting to its akinetic effects over a comparable dose range in both rats and mice (7,25,35,41,42,60).

In a pilot study to the present series, the effects of 0.5–5.0 mg/kg quinpirole on behaviour in the plus-maze were assessed. That study confirmed the potent cataleptic-like effects of this compound in DBA/2 mice (6,42,44) in that even the lowest dose tested produced profound immobility. The results reported here relate to the follow-up study that employed a

10-fold lower dose range (0.06–0.5 mg/kg). These data show that, at 0.25–0.5 mg/kg, quinpirole reduced total arm entries and rears, and markedly enhanced nonexploratory behaviour. Head dipping and total stretched-attend postures were more sensitive to the inhibitory effects of the drug, with reductions evident across the entire dose range. Although an increase in percent open-arm entries was observed at the highest dose tested, the massive behavioural suppression evident at this dose (immobile for > 50% test session) coupled with the absence of any other significant alterations in anxiety measures, would suggest an explanation in terms of behavioural nonspecificity.

The observed locomotor profile of quinpirole is consistent with many other reports in both rats and mice (2,6,16, 38,41,42,44,57,61). Although present results also agree well with reports that, over a comparable dose range, quinpirole is without effect in both rat (29) and mouse (2) plus-maze tests, they are at variance with work that suggests that this D_2 receptor agonist enhances anxiety-related behaviour in mice. More specifically, it has consistently been shown that quinpirole induces hyperdefensiveness towards nonaggressive partners in previously defeated male mice (2,3,6,41,44). As this effect is much less obvious in DBA/2 mice vs. other strains (6,44), it could be argued that the present failure to find anxiogenic-like effects with quinpirole relates to the mouse strain employed. However, this explanation would not account for the reported enhancement of flight behaviour by quinpirole in resident-intruder interactions in male DBA/2 mice (19). It might then be argued that the difference between positive and negative studies relates to specifics of the test situation. Thus, where increased defensiveness has been seen, this has occurred in a social context, whereas the plus-maze is a solitary test. As Gao and Cutler (19) have reported, anxiogenic-like effects with quinpirole in the mouse light–dark exploration test (another solitary model), this interpretation also fails to account for the data. As such, one is left wondering about the possible contribution of the undisputed motoric effects of quinpirole to its apparent anxiogenic-like profile in certain tests.

In contrast to the above findings with SKF 38393, SCH-23390, and quinpirole, our results with sulpiride provide clear evidence of an alteration in anxiety-related behaviour. In the absence of changes in total entries, rearing and nonexploratory behaviour, sulpiride displayed an unambiguous anxiolytic-like profile. More specifically, the compound produced dose-dependent reductions in anxiety, as measured by the traditional indices of percent open-arm entries and percent open-arm time. This profile was confirmed by the novel ethological measures taken. Thus, a benzodiazepine-like effect (9) was observed on exploratory head dipping, with an increase in total dipping accompanied by a reduction in the display of this behaviour from relatively secure areas of the maze. Although total stretched-attend postures were unaffected by sulpiride, the proportion displayed from secure locations (%pSAP) was significantly reduced at 5.0–20.0 mg/kg. As higher doses were required to alter percent open entries and percent open time, the latter finding confirms the sensitivity of risk assessment measures to changes in anxiety state in the maze. The failure of sulpiride to alter (i.e., reduce) another risk assessment measure, closed-arm returns, can simply be explained by the very low control levels in the present study.

Together, present data confirm (and extend to the murine elevated plus-maze) earlier reports of anxiolytic-like effects following sulpiride treatment in rat punished drinking and light–dark tests (40), the mouse light–dark test (12), and the mouse hyperdefensiveness test (41). Furthermore, the absence of motor impairment with this compound agrees well with reports from other laboratories (12,40–42,46). However, the profile of sulpiride differs markedly from that of haloperidol in the same test: Cole and Rodgers (10) found that even very low doses of haloperidol (0.01 mg/kg) produce profound behavioural disruption on the maze without significantly altering any anxiety measure. The difference between these two D_2 receptor antagonists must, therefore, be due to differential affinities for the D_1 receptor, the greater selectivity of sulpiride for mesolimbic/mesocortical D_2 receptors, and/or the relative effects of these agents on other (i.e., D_3 and D_4) receptor populations (20,32,63).

In summary, the present ethopharmacological study suggests that the type of anxiety displayed by mice in the elevated plus-maze test probably does not involve dopamine D_1 receptor mechanisms: a partial agonist at this site, SKF 38393, was behaviourally inert while the selective antagonist, SCH-23390, reduced global locomotion and directed exploration. The selective anxiolytic-like profile obtained with sulpiride would favor the involvement of D_2 receptor mechanisms in plus-maze anxiety. However, the nonspecific profile obtained with the D_2 agonist, quinpirole, and markedly contrasting effects of sulpiride and haloperidol in this test, combine to suggest the need for further study using ligands more selective for D_2, D_3, and D_4 sites.

ACKNOWLEDGEMENTS

E.M.N. was supported by a Royal Society Kapitza Fellowship, and J.C.C. by the Medical Research Council.

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