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Behavioural Profile in the Chicken of CQ 32-084 and CQP 201-403, Two Dopamine Agonists

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FERRARI, F., F. PELLONI AND D. GIULIANI. *Behavioural profile in the chicken of CQ 32-084 and CQP 201-403, two dopamine agonists.* PHARMACOL BIOCHEM BEHAV **45**(1) 117–122, 1993.—CQ 32-084 and CQP 201-403, two ergot derivatives that previous behavioural studies in rats had suggested to be differently active on dopamine (DA) receptors, were IP injected into male chickens. Both compounds strongly modified the animals' behaviour. CQ 32-084 led to sedation, increased yawning, and decreased preening, while CQP 201-403 exerted a biphasic activity: At a low dose, it elicited sedation and yawning; at high doses, however, it induced a state of excitation manifested by diminished sedation and yawning, enhanced preening, and pecking. The sedation, increased yawning, and decreased preening induced by the two DA agonists were reversed by the D_2-selective antagonist, sulpiride. The present studies indicate that, from a behavioural point of view, chickens respond similarly to rats to the DA agonists CQ 32-084 and CQP 201-403, which differ in their selectivity of action on the various DA receptor subtypes.

CQ 32-084 CQP 201-403 Dopamine receptors Chickens Yawning Sedation Preening Pecking

DOPAMINE (DA) agonist-induced behavioural effects have been widely investigated in rodents but only rarely in avians despite the fact that catecholamines are present in high concentrations in their CNS (20).

Early studies clearly implicate DA receptor activation in the stereotyped pecking elicited by the mixed D_1/D_2 agonist, apomorphine (5,6), in accordance with the results obtained in rats, where apomorphine at high doses typically induces stereotyped behaviour (SB). However, since the discovery of the two DA receptor subtypes, D_1 and D_2 (21,32), several forms of rat behaviour besides SB, which seems to depend upon D_1/D_2 synergy (4,23,35), have been identified that are able to distinguish between specific D_1 and D_2 activation. It is accepted, for example, that grooming is increased by D_1-selective agonists (25,31) but inhibited by D_2 agonists (14–16,27); it is also well documented that sedation (11) and a typical stretching-yawning (SY) syndrome (11,13,24,29,33,34) are induced by all D_2 stimulants, at least over a certain dose range. The latter two behavioural effects are visible after the administration of DA agonists at doses too low to elicit SB and also after selective D_2 autoreceptor agonists (11,24); moreover, they are suppressed by pretreatment with specific D_2 autoreceptor antagonists (27). It has therefore been proposed that they are the expression of selective activation of D_2 autoreceptors (11,27,33,34), presynaptically located (21) or, alternatively, of particular D_2 postsynaptic receptors, which are similar to autoreceptors in their sensitivity to DAergic drugs (24,29).

The present experiments were performed to compare the behavioural profile in chickens of two ergot derivatives described as having an agonistic influence on DA receptors (17) and as inducing distinct behavioural effects in rats (12), thus suggesting a different underlying affinity for the DA receptor subtypes.

METHOD

The subjects were 8- to 10-week-old male Sever chickens (Morini, S. Polo d'Enza, Reggio Emilia, Italy) weighing 500–700 g. They were housed in groups of 10, with food and water ad lib and on a 12 L : 12 D cycle (light 7:00 a.m.–7:00 p.m.) for at least 1 week prior to the start of the tests.

All the tests were performed between 9:00 a.m. and 1:00 p.m. in a soundproof, air-conditioned room, and the animals were observed by experimenters unaware of the experimental design. Each chicken was used only once and the controls were handled similarly to the treated animals (no less than eight for each treatment group). Immediately after the IP injection of CQ 32-084, CQP 201-403, or saline, the animals were transferred in pairs to glass observation cages (40 × 30 × 34 cm). The test started 10 min later and lasted 30 min, no less than four experiments being performed for each treatment

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group. Observers were positioned behind a one-way mirror, itself 60 cm from the cage, to avoid eye contact between them and the subjects. Numerous studies have in fact shown that chickens have an aversion to the direct gaze of the experimenter.

The study was conducted in two successive periods. In the first series of experiments, chickens received the two DA agonists (Experiment 1). On the same day, two separate groups of observers scored the pairs of animals treated with saline, CQ 32-084, and CQP 201-403 at all the doses reported in the Results section. On the following days, over a period of about 10 days, the experiments were repeated, varying both the order of treatments and the group of observers for the two compounds. In the second series, chickens received the D_2 antagonist, sulpiride, before the two DA agonists. In this case, all chickens received two consecutive treatments. As before, the pairs of chickens were selected at random from the same housed group and received the same treatment.

Because the group injected with saline and the DA agonists (Experiment 2) did not differ significantly from those injected with the DA agonists alone (Experiment 1), Experiments 1 and 2 are described together in the Results section even though they are presented separately (Figs. 1–4, Experiment 1; Fig. 5, Experiment 2) for clarity.

The following aspects of behaviour were examined: yawning, sedation, preening, and pecking. Apart from yawning, which was scored for each animal throughout the observation period, the signs were evaluated according to the method proposed by Gispen et al. (18) for grooming, with slight modifications. In brief, at 15-s intervals an observer recorded whether or not each animal displayed sedation, preening, or pecking; if one of them was observed, one point (or two in the case of sedation, see below) was recorded for the behaviour in question. Sedation scored one point when the animal, often perching, appeared completely immobile with hooded gaze and two points when its eyes were closed. Preening was counted as such when the chicken drew its feathers through its beak; finally, pecking was scored when the chicken pecked at the walls or floor of the cage or at the second animal.

Drugs and Treatments

The following substances were used: CQ 32-084 and CQP 201-403 (Sandoz, Basel, Switzerland); L-sulpiride (Ravizza Milan, Italy). The drugs were freshly dissolved in saline at concentrations that allowed the IP administration of 1 ml/kg. Doses of drugs were chosen on the basis of previous experiments on rats and are reported in the figures. Sulpiride was injected 15 min before CQ 32-084 and CQP 201-403.

Statistical Evaluation

Data are presented as means ± SEM and analyzed using analysis of variance (ANOVA) followed by Student-Newmann-Keuls test, Student's *t*-test, Kruskal-Wallis followed by Mann-Whitney's *U*-test, and Mann-Whitney's *U*-test, where appropriate. The level of significance was set at $p < 0.05$.

RESULTS

As seen in Figs. 1a and 1b, in the 30-min observation period all control chickens recorded a fair number of yawns. CQ 32-084 at 0.5 and 1 mg/kg significantly increased the phenomenon while CQP 201-403 was effective only at the lowest dose (0.01 mg/kg); at higher doses, yawning was unaffected or eventually reduced until at the highest dose (5 mg/kg) it was completely suppressed (Fig. 1b). The behavioural pattern obtained after the dose of 0.01 mg/kg differed significantly not only from that of the controls but also from that of all the other treatment groups (Fig. 1b). The yawning enhancement induced by CQ 32-084 (1 mg/kg) and CQP (0.01 mg/kg) was antagonized by pretreatment with the D_2 antagonist sulpiride (20 mg/kg) (Fig. 5a).

The effects on sedation (Fig. 2) paralleled those on yawning; marked sedation appeared after the same doses of CQ 32-084 (Fig. 2a) and CQP 201-403 (Fig. 2b) that contemporaneously increased yawning (Fig.1), and both completely disappeared after CQP 201-403 at 5 mg/kg. A significant antagonism of the sedation was also obtained after CQP 1 mg/kg (Fig. 2b). Once again, sulpiride pretreatment (20 mg/kg) abol-

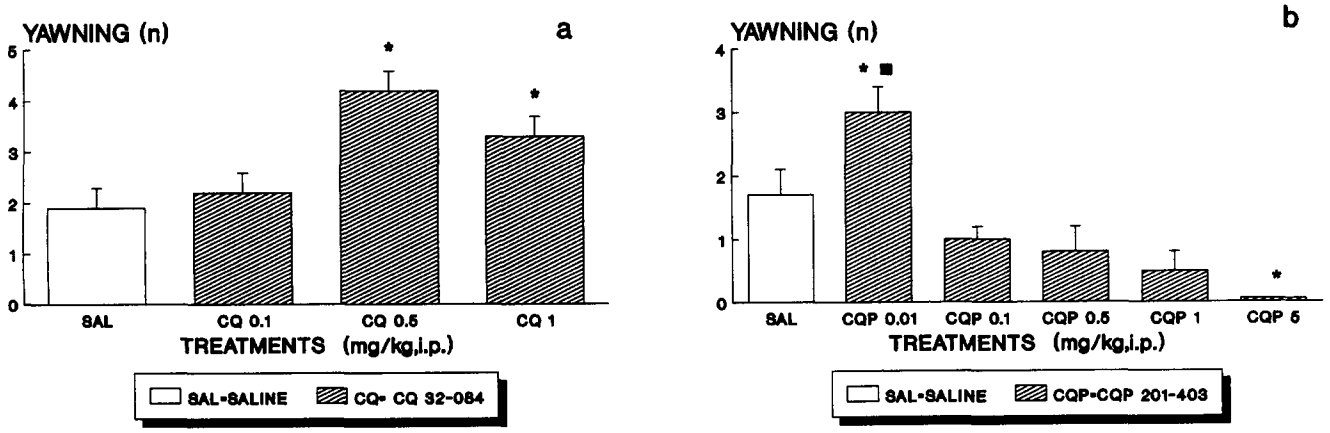

FIG. 1. Effect of CQ 32-084 and CQP 201-403 on yawning in chickens. CQ 32-084 (CQ) (a) and CQP 201-403 (CQP) (1b) or saline (SAL) were IP injected 10 min before the observation period (30 min). Each histogram represents the mean ± SEM of the values per treatment group. Number of chickens for treatment group: SALCQ = 16; CQ 0.1 = 10; CQ 0.5 = 10; CQ 1 = 10; SALCQP = 16; CQP 0.01 = 8; CQP 0.1 = 8; CQP 0.5 = 10; CQP 1 = 10 and CQP 5 = 8. (*) Significantly different from respective controls (ANOVA followed by SNK-test). (■) Significantly different from higher doses (ANOVA followed by SNK-test).

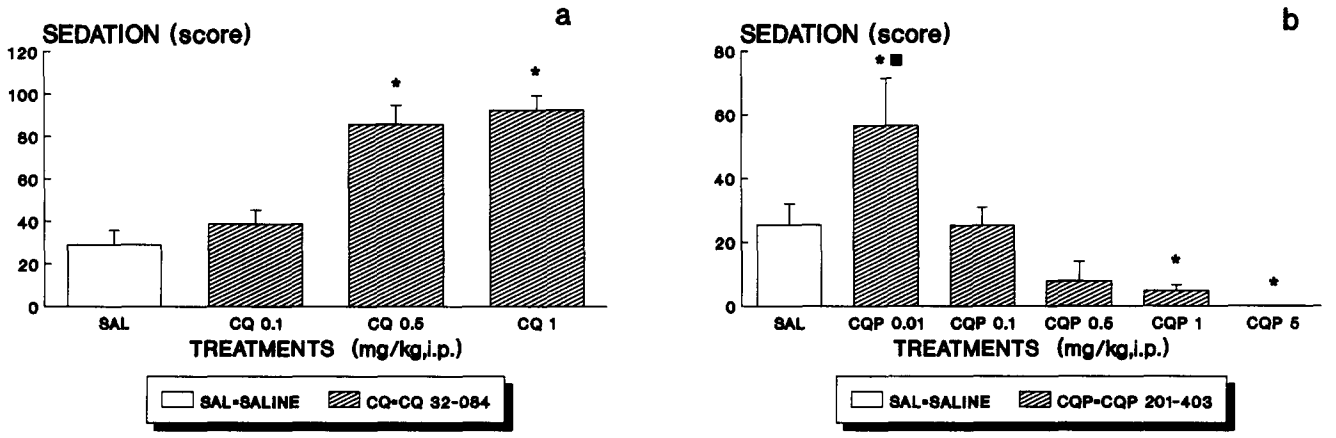

FIG. 2. Effect of CQ 32-084 and CQP 201-403 on sedation in chickens. Legend as in Fig. 1. (*) Significantly different from respective controls (Kruskal-Wallis test followed by Mann-Whitney's *U*-test). (■) Significantly different from higher doses (Kruskal-Wallis test followed by Mann-Whitney's *U*-test).

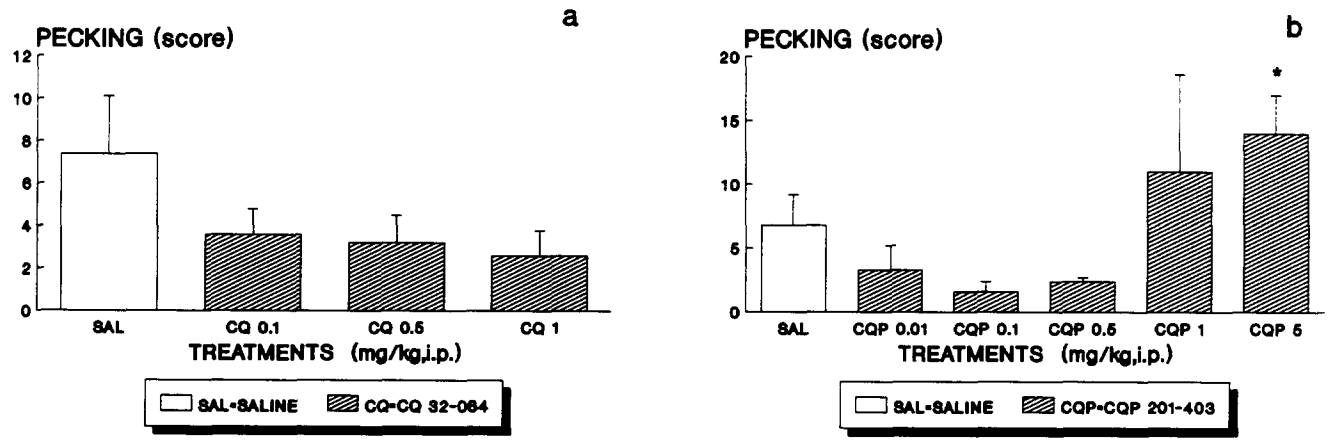

FIG. 3. Effect of CQ 32-084 and CQP 201-403 on pecking in chickens. Legend as in Fig. 1. (*) Significantly different from respective controls (Kruskal-Wallis test followed by Mann-Whitney's *U*-test).

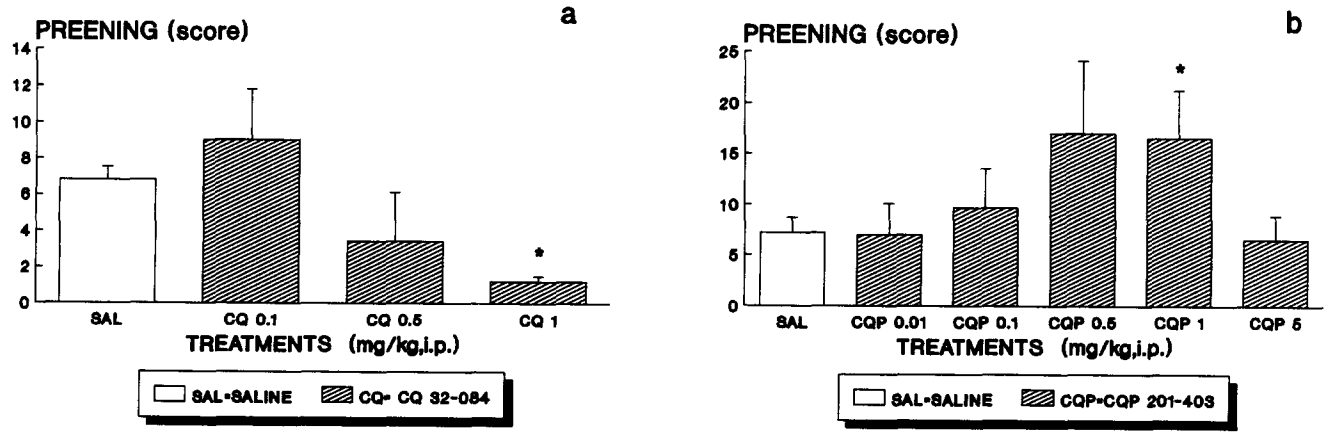

FIG. 4. Effect of CQ 32-084 and CQP 201-403 on preening in chickens. Legend as in Fig. 1. (*) Significantly different from respective controls (Kruskal-Wallis test followed by Mann-Whitney's *U*-test).

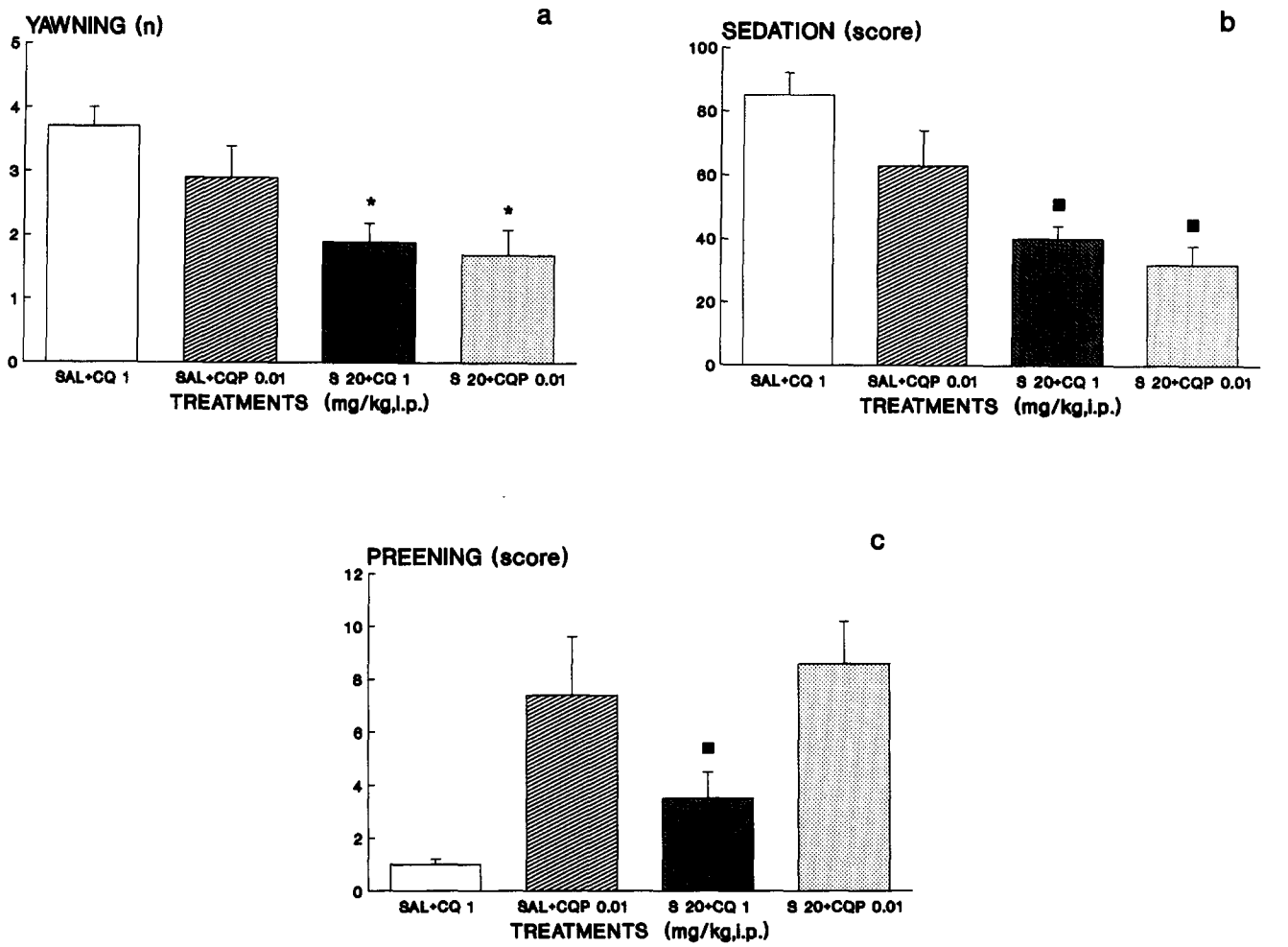

FIG. 5. Influence of sulpiride on the effects produced by CQ 32-084 and CQP 201-403 on yawning, sedation, and preening of chickens. Sulpiride (S) and saline (SAL) were IP administered 15 min before CQ 32-084 (CQ) and CQP 201-403 (CQP). Each histogram represents the mean ± SEM of the values per treatment group. Number of chickens for treatment group = 8. (*) Significantly different from respective controls (Student's *t*-test). (■) Significantly different from respective controls (Mann-Whitney's *U*-test).

ished the increment in sedation provoked by CQ 32-084 (1 mg/kg) and CQP 201-403 (0.01 mg/kg) (Fig. 5b).

Pecking behaviour (Fig. 3) varied considerably among the animals, so no significant result was obtained except an increase after CQP 201-403 at 5 mg/kg (Fig. 3b). For this reason, the behaviour in question was not scored in the experiments with the D_2 antagonist sulpiride.

Figure 4a shows that preening was reduced by CQ 32-084 at 1 mg/kg; CQP 201-403, on the other hand, gradually increased this sign (Fig. 4b), although, once again, the variability in response, already observed for pecking and reported by other authors (9), meant that no significance could be read into the results except at the dose of 1 mg/kg (Fig. 4b). At the highest dose of CQP 201-403 (5 mg/kg), preening reverted to levels similar to those of the controls.

When sulpiride (20 mg/kg) was injected before CQ 32-084 (1 mg/kg) and CQP 201-403 (0.01 mg/kg), it reversed the inhibition of preening caused by CQ 32-084 (Fig. 5c).

DISCUSSION

Our findings clearly show that, in chickens, the DA agonists, CQ 32-084 and CQP 201-403, produce distinct behavioural effects according to receptor selectivity and in almost perfect agreement with the results obtained in rats (12). The two compounds examined are ergot derivatives originally described as rather selective D_2 agonists (17). Subsequent behavioural studies in rats suggested that they must differ profoundly in their specific neurochemical activity because they elicit different behavioural patterns. Whereas CQ 32-084 only increases SY and leads to sedation in the form of hypomotility over a wide dose range, CQP 201-403 exhibits a biphasic activity: SY and sedation are apparent after low doses and disappear when the dosage increases, to be replaced by SB and shaking (12).

It would therefore appear that CQ 32-084 selectively stimulates D_2 autoreceptors or "autoreceptor-like receptors," whereas CQP 201-403 behaves like classic DA agonists, such as apo-

morphine, which acts both at pre- and postsynaptic levels, on D_2 and D_1 receptors with a preferential affinity for D_2 autoreceptors at low doses. In both rats and chickens, certain behavioural signs seem to be particularly sensitive pointers to specific DAergic activity. Naive chickens, like other avians (28), yawn spontaneously and it must be admitted in the light of our experience that, under identical experimental conditions, chickens yawn more that rats (13). Nevertheless, our present work shows that chickens, like rats, respond to D_2 agonist injection (possibly through D_2 autoreceptors) with a significant increase in yawning that is antagonized by the D_2 blocker sulpiride. In both species, moreover, yawning is accompanied by induced sedation, which is also counteracted by sulpiride. As regards these two signs, therefore, CQ 32-084 and CQP 201-403 replicated in chickens what had already been observed in rats, suggesting a divergence in their neurochemical activity that was confirmed by the results on preening. In fact, in the present experiments preening was significantly inhibited by CQ 32-084 and enhanced by CQP 201-403. Preening in avians is comparable to grooming in rats, grooming being a normal trait of the rodents' behaviour that serves different physiological and ethological functions (3,19), and is more marked during mildly stressful events (2,19).

It has long been recognized that DA plays a central role in modulating rat grooming (7,19,30). While DA D_1 agonists and antagonists respectively enhance and antagonize grooming (25,31), some D_2 agonists inhibit it (15,33), in particular at the low doses that preferentially stimulate D_2 autoreceptors. A key role of this subclass of receptors is supported by the potent antagonism exerted on grooming by B-HT 920 (15), which has been described as a selective D_2 autoreceptor agonist (1,26). In view of the recognized interaction between D_1 and D_2 receptor systems for the regulation of DAergic behaviour (25,35), it is possible that reduced DAergic transmission by B-HT 920 attenuates or suppresses some D_1-mediated behaviours, such as grooming.

Experiments in avians indicate that dopaminergic mechanisms are also involved in preening (8) and that the D_1/D_2 agonist apomorphine modulates this behaviour in a biphasic manner, similarly to CQP 201-403 (9). It has been described that the sequence pecking–preening–yawning and sedation is visible in the avian species in response to a stressing stimulus, the signal of the return to normal after arousal being represented by yawning (8). This is in perfect accord with the proposed key role of yawning and D_2 autoreceptors in homeostatic brain mechanisms (14,22,28). It is interesting that the behavioural sequence described above was obtained, in reverse, with the administration of increasing doses of CQP 201-403, which is not a selective stimulant of DA receptor subtypes: At low doses, the compound sedated chickens and induced yawning; at higher doses, this gave way to increased excitation as manifested by preening and pecking; finally, in a state of full arousal in which sedation and yawning were absent chickens adopted an erect posture, displaying their tail feathers and clucking.

In conclusion, the results obtained in chickens confirm what has been observed in rats, namely, that the two DA agonists CQ 32-084 and CQP 201-403 differ in their specific neurochemical activity on DA receptor subtypes; moreover, our data suggest that some simple, fundamental behavioural expressions, such as yawning, grooming, sedation, and excitation, are similarly modulated by DAergic transmission in different species.

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