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Drugs acting at D–1 and D–2 dopamine receptors induce identical purposeless chewing in rats which can be differentiated by cholinergic manipulation

P. Collins[1], C.L.E. Broekkamp[2], P. Jenner[1], and C.D. Marsden[1]*

[1] Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London SW3 6LX, UK
[2] CNS Pharmacology Department, Organon, NL-Oss, The Netherlands

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Abstract. Purposeless chewing in rats was dose dependently increased by acute administration of the dopamine D–1 receptor agonist SKF 38393 (5–20 mg/kg), the D–2 receptor antagonist sulpiride (10–100 mg/kg) and the D–2 receptor agonist quinpirole (0.05–0.25 mg/kg). Only high doses of the D–1 receptor antagonist SCH 23390 (1 and 5 mg/kg) induced purposeless chewing. SCH 23390 (0.05 mg/kg) blocked SKF 38393 (20 mg/kg)-induced purposeless chewing, but had no effect on the purposeless chewing induced by sulpiride (100 mg/kg) or quinpirole (0.1 mg/kg). A dose of SKF 38393 (5 mg/kg) which did not itself induce chewing, potentiated the increase in purposeless chewing observed after administration of sulpiride (100 mg/kg). Administration of SKF 38393 (20 mg/kg) and quinpirole (0.1 mg/kg) did not induce purposeless chewing but stereotyped licking was observed. Administration of sulpiride (100 mg/kg) with quinpirole (0.1 mg/kg) produced an incidence of purposeless chewing not different from that observed when either compound was administered alone. Acute administration of the cholinergic agonist pilocarpine (0.5–4.0 mg/kg) or the cholinesterase inhibitor physostigmine (0.05–0.2 mg/kg) increased the frequency of purposeless chewing in rats. Co-administration of pilocarpine (0.5 mg/kg) with sulpiride (100 mg/kg) increased the frequency of purposeless chewing above that seen when either compound was administered alone. Co-administration of pilocarpine (0.5 mg/kg) with SKF 38393 (20 mg/kg) increased the frequency of purposeless chewing in an additive manner. Co-administration of physostigmine (0.1 mg/kg) with sulpiride (100 mg/kg) but not SKF 38393 (20 mg/kg), increased the frequency of purposeless chewing above that observed when either compound was administered alone. Quinpirole (0.1 mg/kg)-induced purposeless chewing was not affected by co-administration with either pilocarpine (0.5 mg/kg) or physostigmine (0.1 mg/kg). The anticholinergic agent scopolamine (0.1 mg/kg) blocked the purposeless chewing induced by either SKF 38393 (20 mg/kg) or sulpiride (100 mg/kg), but had no effect on the purposeless chewing induced by quinpirole (0.1 mg/kg). Contrary to previous reports, acute manipulation of D–1 or D–2 receptor function can both enhance purposeless chewing behaviour in rats. These apparently identical behaviours can be differentiated by the response to cholinergic manipulation.

Key words: Purposeless chewing – D–1 receptors – D–2 receptors – Cholinergic manipulation – acute dystonia

Neuroleptic administration is associated with a high incidence of involuntary movements (dyskinesias). Attempts at inducing these abnormal movements in rats have been partially successful. Rats administered neuroleptics do not show alterations in posture, but do show an increased incidence of purposeless or vacuous chewing movements (Clow et al. 1980; Waddington et al. 1983), which are similar to the oral-buccal-masticatory movements classically described in patients with tardive dyskinesia.

The relative involvement of D–1 and D–2 receptors in the induction of purposeless chewing has not been resolved. Rosengarten and colleagues (1983) reported that acute administration of the D–1 agonist SKF 38393 induced repetitive jaw movements in rats, which were potentiated by pretreatment with the selective D–2 antagonist spiperone. These data suggested that purposeless chewing was modulated by D–1 receptors over which D–2 receptors exert an opposing action. Subsequently, this conclusion was supported by the findings of others (Johannson et al. 1987; Murray and Waddington 1989a). However, other results conflict with the proposed relationship between the D–1 and D–2 receptors and purposeless chewing. Using a range of D–1 agonists Murray and Waddington (1989a) were unable to induce purposeless chewing in rats. It has also been reported that both

* *Present address:* Department of Clinical Neurology, Institute of Neurology, National Hospital, Queen Square, London WC1, UK

Offprint requests to: P. Jenner

selective and non-selective D–1 and D–2 antagonists can induce purposeless chewing (Waddington et al. 1983; Rupniak et al. 1985). Indeed, the ability of neuroleptic drugs alone to induce this behaviour may complicate the interpretation of the effects of neuroleptics on D–1 agonist induced chewing. Lastly, administration of D–2 agonists may also induce purposeless chewing or similar syndromes (Longoni et al. 1987; Murray and Waddington 1989b).

An alternative explanation might be that both D–1 and D–2 systems contribute individually to the production of an identical behavioural syndrome of purposeless chewing. The response of D–1 and D–2 receptor mediated purposeless chewing to cholinergic manipulation might distinguish between such behaviours, since D–1 and D–2 receptors appear to produce opposing or independent effects on cholinergic neurones in striatum (Fage and Scatton 1986; Gorell et al. 1986; Consolo et al. 1987). Indeed, it has been suggested that D–1 mediated cataleptogenic and antistereotypic actions of neuroleptics may be insensitive to scopolamine reversal, in contrast to the production of these behaviours by D–2 selective antagonists (Arnt et al. 1986; although see Ogren and Fuxe 1988 and Undie and Friedman 1988).

We now report the effects of selective D–1 and D–2 agonist and antagonist drugs on the incidence of purposeless chewing in rats and the response to cholinergic manipulation. Using a limited range of doses, we suggest that both D–1 and D–2 receptors can induce and modulate this behaviour, but the results of cholinergic manipulation suggest that D–1 and D–2 receptors may mediate purposeless chewing by different mechanisms.

Materials and methods

Animals. Male Wistar rats 250–350 g (Bantin and Kingman Ltd, Hull) were used in all experiments. Rats were housed six to a cage and allowed free access to rat chow and tap water. The animals were maintained on a 12-h light/dark cycle, 7.00–19.00, at a temperature of $20 \pm 1°$ C with a relative humidity of $60 \pm 5\%$. All experiments were conducted between 10.00 and 17.00 hours. On the day prior to the experiment the animals were removed from the rat colony and kept overnight in the behavioural testing area to acclimatize them to the environment and to ensure minimal stress immediately prior to the experiments. In some studies animals were used on more than one occasion, but never more than twice and with at least 14 days drug-free interval between experiments.

Assessment of perioral behaviours. Animals were placed in individual plastic cages, with a floor area of 23×18 cm^2 bounded on three sides and with the front open to allow clear observation. Exploratory behaviour ceased within 5–10 min of placement within the observation cages, so allowing uninterrupted observation of the head and mouth. Perioral behaviours were observed during 5-min observation periods.

Characterization of perioral behaviours. The perioral behaviours were characterized as follows. *Purposeless chewing* was observed as a rapid repetitive movement of the lower jaw, which resembled chewing but was not directed toward any particular object. The behaviour occasionally included protrusion of the tongue, and occurred sometimes prior to, or shortly after, tremulous movements of the cheek musculature. *Yawning* was observed as gradual mouth opening followed by momentary retention of the fully opened mouth and more rapid closure. As such, this was easily distinguished from purposeless chewing.

Effect of dopaminergic drugs on perioral behaviours. The ability of each compound to induce purposeless chewing was assessed initially in dose response studies. Following drug administration, the rats were immediately placed into the test areas and observed for 5-min periods at intervals throughout the duration of the experiment. Rats were observed every 15 min for 1 h and again at 2 h following SKF 38393 (5–20 mg/kg) administration, after 30 min and every hour for 3 h after sulpiride (10–100 mg/kg) administration, every 15 min for 1 h after SCH 23390 (0.1–5.0 mg/kg) administration, and every 15 min for 45 min after quinpirole (0.05–0.25 mg/kg) administration.

Effect of combined administration of dopaminergic drugs on perioral behaviour. For studies in which dopaminergic compounds were co-administered, the dose and time after administration which produced the maximum increase in purposeless chewing were used unless otherwise specified. These were as follows: SKF 38393 20 mg/kg, 30 min; sulpiride 100 mg/kg, 30 min; Quinpirole 0.1 mg/kg, 15 min. In combined administration experiments, each compound was administered independently from separate syringes into opposite sides of the abdomen.

Effect of SKF 38393 and sulpiride on purposeless chewing induced by drugs acting on D–2 receptors. SKF 38393 (20 mg/kg and 5 mg/kg) and sulpiride (100 mg/kg) were administered 30 min before observation. Quinpirole (0.1 mg/kg) was administered 15 min before observation.

Effect of SCH 23390 on purposeless chewing induced by drugs acting on D–1 and D–2 receptors. SCH 23390 (0.05 mg/kg) was administered simultaneously with SKF 38393 (15 min before observation), sulpiride (30 min before observation) and quinpirole (15 min before observation).

Effect of cholinergic drugs on perioral behaviour. The ability of each compound to induce purposeless chewing was assessed initially in dose response studies. Following drug administration, the rats were immediately placed into the test cages and observed for 5-min periods at intervals throughout the duration of the experiment. Rats were observed every 5 min for 30 min following administration of pilocarpine (0.5–4.0 mg/kg) and every 5 min for 45 min following physostigmine (0.05–0.2 mg/kg) administration.

Effect of cholinergic modulation on purposeless chewing induced by drugs acting on D–1 and D–2 receptors. Doses of pilocarpine and physostigmine that induced submaximal increases in purposeless chewing were used for cholinergic modulation experiments. These were as follows: pilocarpine 0.5 mg/kg 15 min before observation and physostigmine 0.1 mg/kg 15 min before observation. Other doses used were SKF 38393 20 mg/kg 30 min before observation, sulpiride 100 mg/kg 30 min before observation, and quinpirole 0.1 mg/kg 15 min before obervation.

Scopolamine (0.1 mg/kg) administered 30 min prior to observation reduced the purposeless chewing produced 30 min after administration of pilocarpine (0.5 mg/kg) without inducing an increase in locomotor activity (data not shown) and was therefore used to assess the effect of cholinergic antagonism of drugs acting on D–1 and D–2 receptors. SKF 38393, sulpiride and quinpirole were administered using the same doses and pretreatment times as above.

Drugs employed. The following drugs were employed: (±)sulpiride (Delagrange), quinpirole hydrochloride (Eli Lilly), SCH 23390 (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benazepin-7-ol maleate (Schering-Plough), SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) hydrochloride (Smith Kline and French), (−)scopolamine hydrobromide, pilocarpine hydrochloride, physostigmine salicylate (Sigma). All

compounds were dissolved in distilled water, except sulpiride which was dissolved in minimal 2% H_2SO_4, diluted to volume with distilled water and adjusted to pH 6.5 with 1 N NaOH. All compounds were administered **IP** at a dose volume of 2 ml/kg body weight.

Statistical analysis. A square root transformation was performed on all purposeless chewing data in order to normalise the variance. Dose response curves were then analysed using a two-way analysis of variance (ANOVA). Where one-way ANOVA demonstrated a significant effect in combination experiments, pairwise *t*-tests on five or nine preplanned comparisons were performed using Dunn's modified *t*-test. Yawning data was analysed using the Mann-Whitney *U* test, with 2% significance which is approximately equal to 5% significance for two comparisons.

Results

In all experiments control animals displayed a low frequency of perioral behaviour occurring as individual chews or as bursts of several chews. Yawning was rarely observed in control animals.

Effect of SKF 38393 on perioral behaviour

Administration of SKF 38393 (5–20 mg/kg) induced a dose-dependent increase in the incidence of purposeless chewing (Fig. 1) [dose effect $F(3,100)=29.1$, $P<0.001$ and time effect $F(4,100)=6.4$, $P<0.001$; two way ANOVA]. The response was apparent 15 min after administration, maximal within 30–60 min and began to subside within 2 h. An increase in sniffing and grooming was also observed, but was not quantified.

Effect of sulpiride on perioral behaviour

Administration of sulpiride (10–100 mg/kg) induced a dose-dependent increase in the incidence of purposeless chewing (Fig. 2) [dose effect $F(3,80)=19.2$, $P<0.001$]. The effect was maximal 30 min after dosing and was maintained for more than 2 h [time effect $F(3,80)=1.5$, NS].

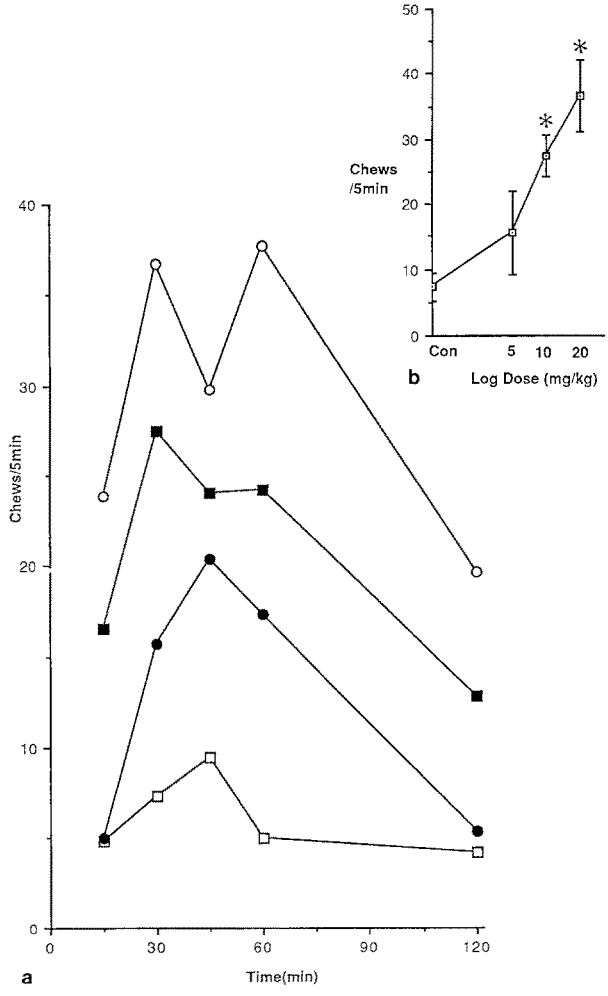

Fig. 1a, b. The time- and dose-dependent induction of purposeless chewing by SKF 38393. **a** The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=6$). □ control, ● 5 mg/kg, ■ 10 mg/kg, ○ 20 mg/kg. **b** The dose dependent increase in purposeless chewing 30 min after administration of SKF 38393 (5–20 mg/kg). One-way ANOVA $F(3,20)=8.1$. $P<0.001$. *$P<0.05$, Dunn's test

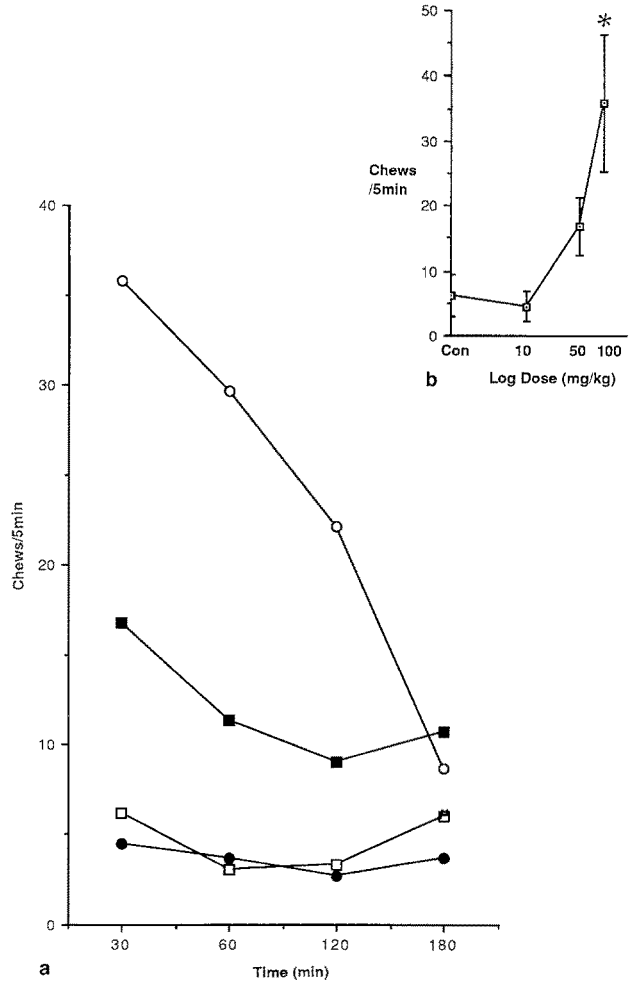

Fig. 2a, b. The time- and dose-dependent induction of purposeless chewing by sulpiride. **a** The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=6$). □ control, ● 10 mg/kg, ■ 50 mg/kg, ○ 100 mg/kg. **b** The dose-dependent increase in purposeless chewing 30 min after administration of sulpiride (10–100 mg/kg). One-way ANOVA $F(3,20)=6.5$. $P<0.01$. *$P<0.05$, Dunn's test

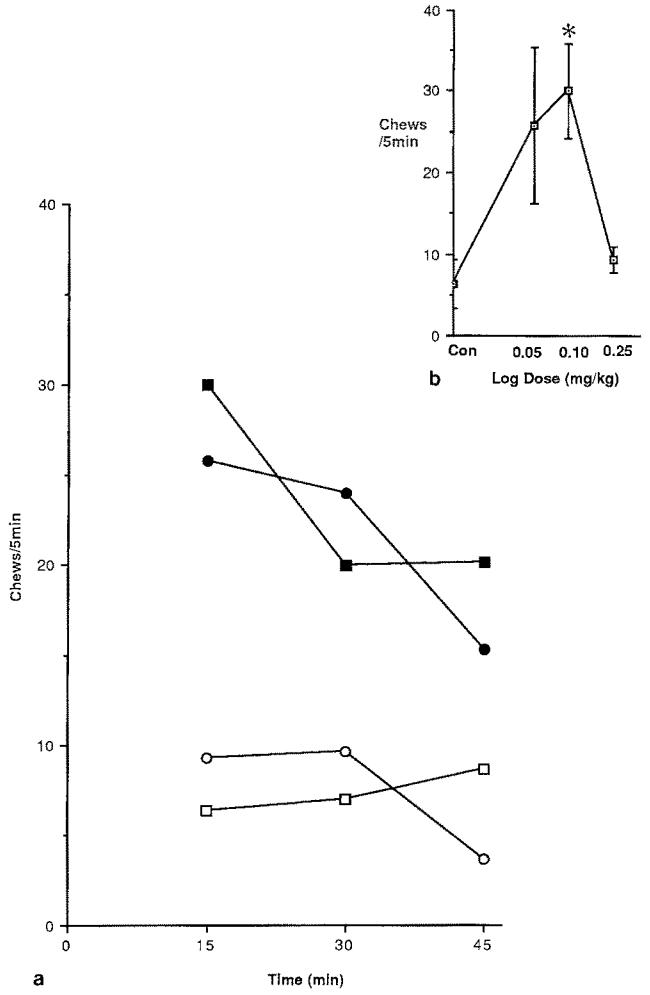

Fig. 3a, b. The time- and dose-dependent induction of purposeless chewing by quinpirole. **a** The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=6$). □ control, ● 0.05 mg/kg, ■ 0.10 mg/kg, ○ 0.25 mg/kg. **b** The dose-dependent increase in purposeless chewing 15 min after administration of quinpirole (0.05–0.25 mg/kg). One-way ANOVA $F(3,20)=6.1$. $P<0.01$. $^*P<0.05$, Dunn's test

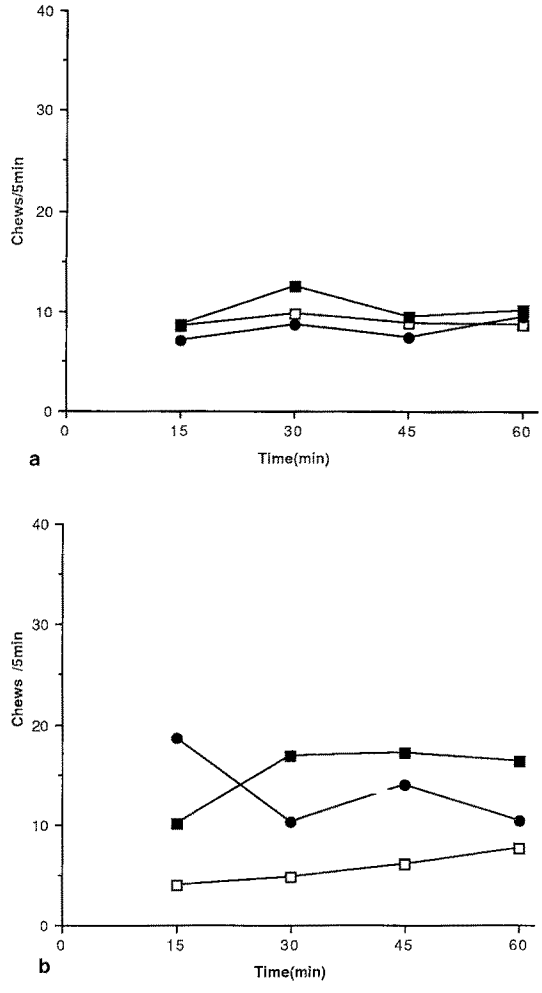

Fig. 4a. The time-dependent induction of purposeless chewing by low doses of SCH 23390. The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=12$). □ control, ● 0.1 mg/kg, ■ 0.5 mg/kg. No differences between control and treated groups were found **b** the time-dependent induction of purposeless chewing by high doses of SCH 23390. The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=6$–12). □ control, ● 1 mg/kg, ■ 5 mg/kg

Effect of quinpirole on perioral behaviour

Administration of quinpirole (0.05 and 0.1 mg/kg) induced an increase in the incidence of purposeless chewing [dose effect $F(3,60)=13.3$, $P<0.001$] (Fig. 3). The effect was maximal within 15 min and was maintained for at least 45 min [time effect $F(2,60)=1.7$, NS]. At a higher dose, 0.25 mg/kg quinpirole induced stereotypic licking and occasional gnawing of cage surfaces but did not increase purposeless chewing above that observed in control animals. Quinpirole induced an increase in yawning frequency (data not shown).

Effect of SCH 23390 on perioral behaviours

Administration of SCH 23390 (0.1 and 0.5 mg/kg) had no effect on purposeless chewing frequency (Fig. 4a). In a separate experiment SCH 23390 (1.0 and 5.0 mg/kg) induced a small but significant increase in the incidence of purposeless chewing [dose effect $F(2,84)=25.3$, $P<0.001$] which was observed within 15 min of administration and remained elevated for at least 1 h [time effect $F(3,84)=1.8$ NS] (Fig. 4b).

Effect of SKF 38393 and sulpiride on purposeless chewing induced by drugs acting on D–2 receptors

Effect of concurrent administration of SKF 38393 and sulpiride. Concurrent administration of sulpiride (100 mg/kg, IP) and SKF 38393 (20 mg/kg, IP) increased the level of purposeless chewing above that observed following administration of either compound alone (although this was significantly increased only when compared to the effect of SKF 38393 alone) (Table 1a). Ad-

Table 1. Effect of SKF 38393 and sulpiride on purposeless chewing induced by drugs acting on D–2 receptors

(a)

Treatment	Vehicle	*n*	Sulpiride (100 mg/kg)	*n*
Control	10.5 ± 2.7	6	26.8 ± 3.8[a]	6
SKF 38393 (20 mg/kg)	23.7 ± 3.4[a]	6	59.2 ± 12.5[a]	6
Control	4.5 ± 1.4	6	26.8 ± 1.4[a]	6
SKF 38393 (5 mg/kg)	7.8 ± 2.9	6	50.4 ± 4.6[a,b]	5

(b)

Treatment	Vehicle	*n*	Quinpirole (0.1 mg/kg)	*n*
Control	3.8 ± 1.8	6	24.2 ± 3.8[a]	5
SKF 38393 (20 mg/kg)	24.8 ± 4.5[a]	6	7.5 ± 3.5[b]	6
Control	5.5 ± 1.6	6	34.3 ± 5.6[a]	6
Sulpiride (100 mg/kg)	22.8 ± 2.4[a]	6	38.2 ± 4.8[a]	6

Data are expressed as the mean number of chews ± 1 SEM (standard error of the mean) over a 5-min observation period
[a] $P<0.05$ compared to control group, Dunn's test
[b] $P<0.05$ compared to treated groups, Dunn's test

Table 2. Effect of SCH 23390 on purposeless chewing induced by drugs acting on D–1 and D–2 receptors

(a)

Treatment	Vehicle	*n*	SCH 23390 (0.05 mg/kg)	*n*
Control	5.8 ± 2.5	6	7.0 ± 2.7	6
SKF 38393 (20 mg/kg)	25.3 ± 2.7[a]	6	8.2 ± 2.1[b]	6
Quinpirole (0.1 mg/kg)	30.2 ± 3.8[a]	6	26.5 ± 3.7[a,c]	6

(b)

Treatment	Vehicle	*n*	SCH 23390 (0.05 mg/kg)	*n*
Control	6.0 ± 2.3	6	9.7 ± 2.9	6
Sulpiride (100 mg/kg)	24.3 ± 3.1[a]	6	26.5 ± 4.1[a,c]	6

Data are expressed as the mean number of chews ± 1 SEM over a 5-min observation period
[a] $P<0.05$ compared to control, Dunn's test
[b] $P<0.05$ compared to SKF 38393-treated group, Dunn's test
[c] $P<0.05$ compared to SCH 23390-treated group, Dunn's test

ministration of a dose of SKF 38393 (5 mg/kg), ineffective when given alone, with sulpiride (100 mg/kg) increased the level of purposeless chewing above that produced by either compound alone (Table 1a).

Effect of concurrent administration of SKF 38393 and quinpirole. Administration of quinpirole (0.1 mg/kg) with SKF 38393 (20 mg/kg) induced a syndrome of stereotypic licking, but did not increase the frequency of purposeless chewing above that observed in control animals (Table 1b).

Effect of concurrent administration of sulpiride and quinpirole. Co-administration of sulpiride (100 mg/kg) and quinpirole (0.1 mg/kg) did not increase the purposeless chewing frequency above that produced by either compound alone (Table 1b).

Effect of SCH 23390 on purposeless chewing induced by drugs acting on D–1 and D–2 receptors. Administration of SCH 23390 (0.05 mg/kg) reduced the purposeless chewing observed after administration of SKF 38393 (20 mg/kg), but had no effect on the level of purposeless chewing induced by quinpirole (0.1 mg/kg) or sulpiride (100 mg/kg) (Table 2a, b).

Effect of cholinergic stimulation on purposeless chewing

Administration of pilocarpine (0.5–4.0 mg/kg) dose-dependently increased the frequency of purposeless chewing [dose effect $F(3,114)=162.2$, $P<0.001$] (Fig. 5). The effect was apparent within 5 min, maximal after 20 min, subsiding thereafter [time effect $F(5,114)=9.3$, $P<0.001$].

Administration of physostigmine (0.05–0.2 mg/kg) also induced a dose-dependent increase in the frequency of purposeless chewing [dose effect $F(3,171)=65.6$, $P<0.001$] (Fig. 6). This effect was again apparent within 5 min, maximal within 20 min, subsiding thereafter [time effect $F(8,171)=3.5$, $P<0.001$].

Effect of cholinergic stimulation on purposeless chewing induced by drugs acting at D–1 and D–2 receptors

Administration of sulpiride (100 mg/kg) with physostigmine (0.1 mg/kg) increased the frequency of purposeless chewing above that observed following administration of either drug alone. However, administration of SKF 38393 (20 mg/kg) with physostigmine (0.1 mg/kg) did not increase the frequency of purposeless chewing above that observed in vehicle treated animals (Table 3a). Combined administration of SKF 38393 (20 mg/kg) and pilocarpine (0.5 mg/kg) increased the frequency of purposeless chewing in an additive manner (Table 3b). Administration of sulpiride (100 mg/kg) with pilocarpine (0.5 mg/kg) increased the frequency of purposeless chewing above that produced by either drug alone (Table 3c). Administration of quinpirole (0.1 mg/kg) with pilocarpine (0.5 mg/kg) or physostigmine (0.1 mg/kg) did not increase the frequency of purposeless chewing above that observed following administration of either drug alone (Table 3d).

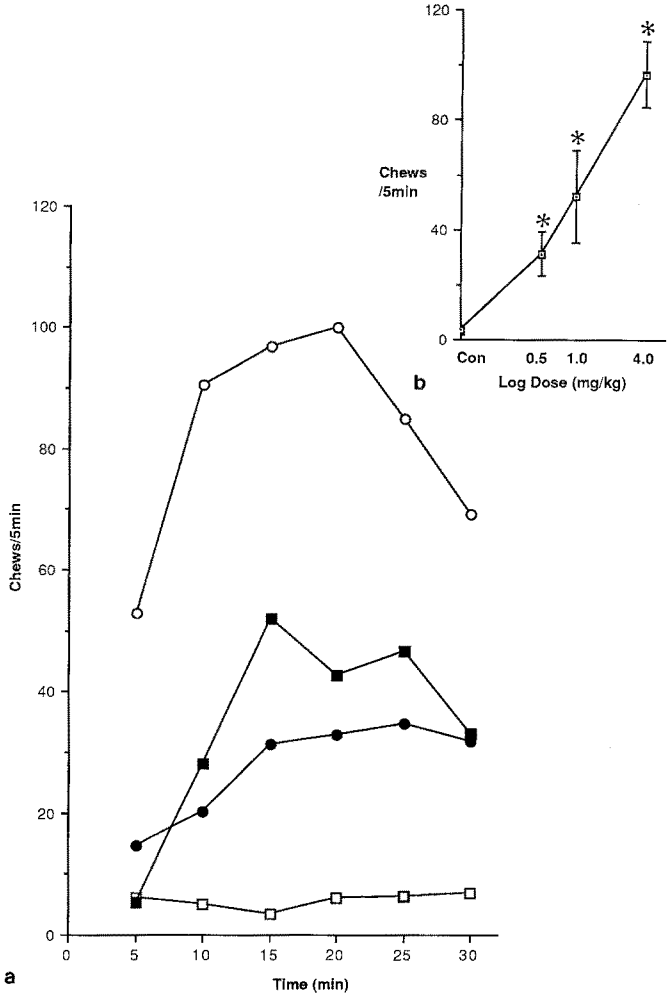

Fig. 5a, b. The time and dose dependent induction of purposeless chewing by pilocarpine. **a** The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=5$–6). □ control, ● 0.5 mg/kg, ■ 1.0 mg/kg, ○ 4.0 mg/kg. **b** The dose-dependent increase in purposeless chewing 15 min after administration of pilocarpine (0.5–4.0 mg/kg). One-way ANOVA $F(3,19)=23.7$. $P<0.001$. $^*P<0.05$, Dunn's test

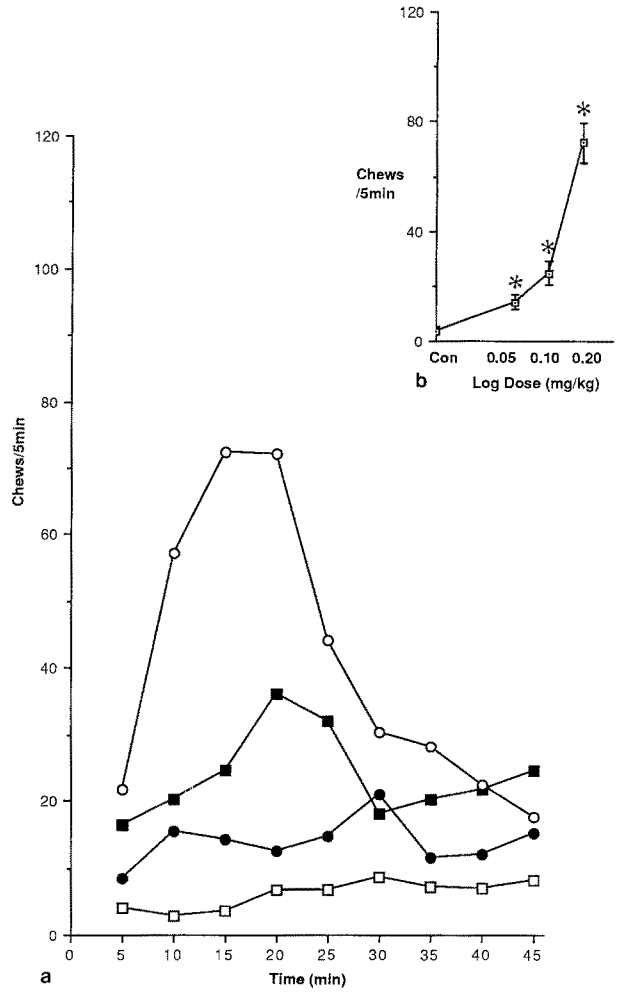

Fig. 6. The time- and dose-dependent induction of purposeless chewing by physostigmine. **a** The points represent the mean number of chews recorded in each 5-min observation period. Error bars are omitted for clarity ($n=5$–6). □ control, ● 0.05 mg/kg, ■ 0.1 mg/kg, ○ 0.2 mg/kg. **b** The dose-dependent increase in purposeless chewing 15 min after administration of physostigmine (0.05–0.2 mg/kg). One-way ANOVA $F(3,19)=36.3$. $P<0.001$. $^*P<0.05$, Dunn's test

Effect of scopolamine administration on purposeless chewing induced by drugs acting on D–1 and D–2 receptors

Administration of the cholinergic antagonist scopolamine (0.1 mg/kg) had no effect on the spontaneous chewing rates in vehicle-treated animals. Scopolamine (0.1 mg/kg) completely blocked the purposeless chewing induced by SKF 38393 (20 mg/kg) and sulpiride (100 mg/kg), but had no effect on the purposeless chewing induced by quinpirole (0.1 mg/kg) (Table 4a). However, quinpirole (0.1 mg/kg)-induced yawning was completely blocked on concurrent administration with scopolamine (0.1 mg/kg) (Table 4b).

Discussion

Purposeless chewing is currently thought to be mediated via the D–1 dopamine receptor, with the D–2 receptor playing an inhibitory role in the modulation of this behaviour (Rosengarten et al. 1983; Johansson et al. 1987). However, since D–2 receptor agonists may also initiate a similar syndrome of chewing (Longoni et al. 1987; Murray and Waddington 1989b), this may not be the case. Indeed, the present study suggests that both D–1 and D–2 receptors may initiate purposeless chewing by separate mechanisms. A summary of the effects of D–1 and D–2 agonists and antagonists on purposeless chewing and the effect of concurrent administration of SCH 23390, sulpiride and quinpirole on these behaviours are presented in Table 5.

The frequency of purposeless chewing in rats was increased following administration of the D–1 agonist SKF 38393 or the D–2 antagonist sulpiride. The rapid purposeless chewing response induced by sulpiride (maximum 30 min) was surprising as it penetrates into brain slowly, and has a long duration of action. However, recent in vivo dialysis studies have shown that sulpiride increases striatal dopamine release with a time course

Table 3. Effect of cholinergic stimulation on purposeless chewing induced by drugs acting on D–1 and D–2 receptors

(a)

Treatment	Vehicle	*n*	SKF 38393 (20 mg/kg)	*n*	Sulpiride (100 mg/kg)	*n*
Control	6.8±1.6	6	27.2±6.3[a]	5	24.7± 3.5[a]	6
Physostigmine (0.1 mg/kg))	18.5±3.1[a]	6	18.0±5.3	6	60.0±12.6[a,b]	6

(b)

Treatment	Vehicle	*n*	SKF 38393 (20 mg/kg)	*n*
Control	4.8±2.0	6	21.5±4.7[a]	6
Pilocarpine (0.5 mg/kg)	29.7±5.2[a]	6	54.3±8.1[a,c]	6

(c)

Treatment	Vehicle	*n*	Sulpiride (100 mg/kg)	*n*
Control	5.8±1.4	6	30.5±2.5[a]	6
Pilocarpine (0.5 mg/kg)	31.0±4.8[a]	6	52.8±4.2[a,b]	6

(d)

Treatment	Vehicle	*n*	Quinpirole (0.1 mg/kg)	*n*
Control	7.5±1.8	6	43.8±9.4[a]	6
Pilocarpine (0.5 mg/kg)	24.8±5.4[a]	6	36.5±4.5[a]	6
Physostigmine (0.1 mg/kg)	31.0±7.6[a]	6	47.5±7.3[a]	6

Data are expressed as the mean number of chews ±1 SEM over a 5-min observation period
[a] $P<0.05$ compared to control group, Dunn's test
[b] $P<0.05$ compared to both treated groups, Dunn's test
[c] $P<0.05$ compared to SKF 38393-treated group, Dunn's test

similar to that reported for purposeless chewing in this investigation (Zetterstrom et al. 1985). The purposeless chewing produced by SKF 38393 and sulpiride was qualitatively identical. The combined administration of SKF 38393 and sulpiride produced a larger response than that observed following the administration of either compound alone. The increased chewing response appears to be a potentiation rather than an additive effect, since an ineffective dose of SKF 38393 was able to enhance the response to sulpiride. These results confirm the earlier findings of Rosengarten and colleagues (1983). The data suggests an interaction between D–1 and D–2 sites in the production of purposeless chewing. They would also appear to support the hypothesis that purposeless chewing is a D–1 mediated phenomenon on which the D–2 receptor is inhibitory. In agreement, Johannson and colleagues (1987) showed that administration of the D–2 agonist quinpirole (0.03–0.3 mg/kg) reduced oral movements in rats.

The ability of SCH 23390 to inhibit SKF 38393 induced purposeless chewing also suggests D–1 involvement in this behaviour. However, the failure of a low dose of SCH 23390 to block sulpiride-induced purposeless chewing suggests that not all purposeless chewing is D–1 mediated. An alternative explanation for the ability of sulpiride to induce purposeless chewing may be that sulpiride induces this behaviour through its actions on D–2 receptors. So, another possibility is that both D–1 and D–2 receptors can initiate purposeless chewing, but that at levels of D–1 stimulation insufficient to induce the behaviour a facilitation of the D–2 response can occur.

In agreement with D–1 agonist activity initiating purposeless chewing, SCH 23390 in doses sufficient to antagonise this behaviour did not itself produce an increase in chewing. However, at higher doses SCH 23390 did induce purposeless chewing. At doses in excess of those required to block D–1 dopamine receptors, SCH 23390 acts on 5HT receptors (Hyttel 1983; Hicks et al. 1984; Bischoff et al. 1986; Skarsfeldt and Larsen 1988). The observation that putative 5HT–1 agonists also can induce purposeless chewing in rats (Stewart et al. 1989) suggests that this may be the mechanism by which high doses of SCH 23390 induce purposeless chewing. Alternatively, the sedation induced by high doses of SCH 23390 may precipitate an increased chewing response, since there is an inverse correlation between oral activity and gross motor behaviour (Levy et al. 1987).

Low doses of the D–2 agonist quinpirole also induced

Table 4a Effect of scopolamine on purposeless chewing induced by drugs acting on D–1 and D–2 receptors

Treatment	Vehicle	*n*	Scopolamine (0.1 mg/kg)	*n*
Control	5.8 ± 2.4	6	7.3 ± 2.2	6
SKF 38393 (20 mg/kg)	25.7 ± 4.4[a]	6	6.2 ± 1.7[b]	6
Control	7.5 ± 2.4	6	5.0 ± 1.6	6
Sulpiride (100 mg/kg)	27.5 ± 5.3[a]	6	7.0 ± 3.0[b]	6
Control	5.5 ± 2.9	6	7.0 ± 3.0	6
Quinpirole (0.1 mg/kg)	23.7 ± 2.8[a]	6	21.3 ± 3.7[a,c]	6

Data are expressed as the mean number of chews ± 1 SEM over a 5-min observation period
[a] $P < 0.05$ compared to control group, Dunn's test
[b] $P < 0.05$ compared to treated group, Dunn's test
[c] $P < 0.05$ compared to scopolamine-treated group, Dunn's test

b Effect of scopolamine on quinpirole-induced yawning

Treatment	Vehicle	Scopolamine (0.1 mg/kg)
Control	0	0
Quinpirole (0.1 mg/kg)	1.8 ± 0.8[a]	0[b]

Data are expressed as the mean number of yawns ± 1 SEM
[a] $P < 0.05$, compared to control group
[b] $P < 0.05$ compared to treated group

Table 5. Summary of the results of dopaminergic modulation of purposeless chewing

	Alone	+ SCH 23390	+ Sulpiride	+ Quinpirole
SKF 38393	↑	↓	↑↑	↓[a]
Quinpirole	↑[b]	↑	↑	
SCH 23390	No effect			
Sulpiride	↑	↑		↑

↑ Stimulation of purposeless chewing
↑↑ Potentiation of purposeless chewing
↓ Blockade of purposeless chewing
[a] Induced stereotypy
[b] Low doses only

an increase in the frequency of purposeless chewing as well as yawning. This behaviour was identical to that produced by sulpiride or SKF 38393. Longoni and colleagues (1987) reported increased yawning in rats treated with quinpirole, accompanied by an unquantified increase in chewing. Murray and Waddington (1989b) showed another D–2 agonist LY 163502 to induce a syndrome of non-stereotypic chewing which was directed toward bedding material and faecal matter, in a non-consummatory manner. This is similar, but not identical to the behaviour we have reported, but our observations were carried out in the absence of bedding. These reports, however, contrast with that of Johansson and colleagues (1987) showing quinpirole to suppress purposeless chewing.

Table 6. Summary of the results of cholinergic modulation of purposeless chewing

	Alone	+ SKF 38393	+ Quinpirole	+ Sulpiride
Pilocarpine	↑	↑[a]	↑	↑↑
Physostigmine	↑	↑(NS)	↑	↑↑
Scopolamine	↓	↓	↑	↓

↑ Stimulation of purposeless chewing
↑↑ Potentiation of purposeless chewing
↓ Blockade of purposeless chewing
NS = Not significant
[a] Additive

At doses in excess of 0.1 mg/kg, quinpirole did not produce purposeless chewing but induced oral stereotypy. This might explain why previously Rosengarten et al. (1983) reported that the D–2 agonist LY 141865 (1 mg/kg) induced stereotypic movements but did not enhance purposeless chewing.

The ability of quinpirole to enhance purposeless chewing was unexpected, considering sulpiride produced the same effect. The inability of SCH 23390 to block quinpirole-induced purposeless chewing suggests the effect is due to an action on D–2 receptors. This further supports the involvement of D–2 receptors in the induction of purposeless chewing. Precisely how both sulpiride and quinpirole can induce purposeless chewing through D–2 mechanisms is not clear. Purposeless chewing produced by a combination of quinpirole and sulpiride was not greater than that produced by either drug alone. This might suggest a common mechanism of action. One possibility would be that the low doses of quinpirole which produced purposeless chewing were acting presynaptically to reduce dopamine function. Quinpirole in combination with SKF 38393 induced stereotyped movements, in agreement with Arnt et al. (1987) and Braun and Chase (1986), whilst SKF 38393 potentiated sulpiride-induced purposeless chewing. Thus, quinpirole and sulpiride may be acting through separate mechanisms, although pre- and post-synaptic actions on D–2 receptors cannot be ruled out.

In an attempt to distinguish between the apparently identical purposeless chewing induced by D–1 and D–2 systems, the response to cholinergic manipulation was investigated. A summary of the effects of cholinergic modulation on the purposeless chewing induced by SKF 38393, quinpirole and sulpiride is presented in Table 6. As previously reported, the directly acting cholinergic agonist pilocarpine or the anticholinesterase physostigmine produced purposeless chewing in the rat which was identical to that observed following administration of the dopamine active drugs (Rupniak et al. 1983; Salamone et al. 1986).

Administration of either pilocarpine or physostigmine with sulpiride produced a large increase in purposeless chewing in excess of that produced by any drug alone. Scopolamine blocked sulpiride-induced purposeless chewing. The effect of these submaximal doses of cholinergic agonist drugs confirms the original findings of Rupniak et al. (1983), which were recently questioned (see

Stoessl et al. 1989). Stewart et al. (1988) previously reported that sulpiride did not affect purposeless chewing induced by pilocarpine (4 mg/kg); however, the use of a submaximal dose of pilocarpine in the present study shows the true nature of this interaction.

Pilocarpine enhanced purposeless chewing induced by SKF 38393 in an additive fashion. The cholinergic antagonist scopolamine inhibited the response to SKF 38393. However, the anticholinesterase physostigmine did not alter the SKF 38393 response, even though both compounds alone produced an increase in purposeless chewing. This might indicate that SKF 38393-induced purposeless chewing is not reliant on cholinergic tone, although drugs acting on cholinergic receptors can modulate this response to SKF 38393.

The difference in response to cholinergic modulation between the purposeless chewing observed following administration of SKF 38393 and sulpiride is particularly interesting. The response of purposeless chewing induced by sulpiride to cholinergic modulation is in agreement with previous work on a selective D–2 antagonist haloperidol (Rupniak et al. 1983). However, Stoessl and colleagues (1989) reported that mouth movements induced by chronic fluphenazine administration could be reduced by SCH 23390 but were unaffected by physostigmine. This profile is consistent with that produced by SKF 38393 in the present experiments, and may suggest that chronic fluphenazine induces mouth movements via a D–1 mediated pathway, so resolving the conflicting results of Rupniak et al. (1983) and Stoessl et al. (1989). However, fluphenazine, which is non-selective for D–1 and D–2 receptors in in vitro experiments, appears to be D–2 selective in vivo in both acute and chronic studies (Arnt and Hyttel 1986; Andersen 1988; McGonicle et al. 1989), so perhaps an alternative explanation is required. For example, there may be differences in the methods used to quantify mouth movements. Rupniak et al. (1983) and this study have focussed on the incidence of purposeless chewing movements, while Stoessl et al. (1989) quantified the total duration of a range of mouth movements. Perhaps separate components of the perioral syndrome respond differently to pharmacological manipulation. For example, the observed effects of chronic neuroleptic treatment on perioral movements are dependent on the experimental conditions (open cage/restraining tube) (Levy et al. 1987)

In complete contrast, purposeless chewing produced by quinpirole was not affected by pilocarpine, physostigmine or scopolamine. This suggests that D–2 agonist-induced purposeless chewing differs in mechanism from that produced following administration of SKF 38393 or sulpiride. This concept is supported by the ability of scopolamine to abolish quinpirole-induced yawning. However, it is stressed that this conclusion is at present based on a single dose of quinpirole.

In conclusion, we have shown that purposeless chewing in rats can be induced by manipulation of D–1 or D–2 receptors and can be differentially modulated by drugs acting on cholinergic receptors. The precise mechanisms through which these drugs induce this behaviour are clearly more complex than has been suggested hitherto, although these interactions need to be confirmed in further investigations utilizing a range of doses of each drug.

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