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Species Differences in Behavioural Effects of Rolipram and Other Adenosine Cyclic 3H, 5H-Monophosphate Phosphodiesterase Inhibitors

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Summary

The effect of the phosphodiesterase (PDE) inhibitors rolipram, Ro 20-1724 and isobutylmethylxanthine (IBMX) on motor behaviour and rectal temperature was studied in mice, rats and guinea pigs following intraperitoneal administration (0.39 to 25 mg/kg). The selective adenosine cyclic 3′, 5′-monophosphate (cAMP) PDE inhibitors rolipram and Ro 20-1724 in each species caused a dissimilar pattern of neurotropic effects: Hypothermia and *hypo*kinesia in mice, hypothermia, *hypo*kinesia and head twitches in rats, hypothermia, *hyper*kinesia and head twitches in guinea pigs. The head twitches were associated with forepaw shaking and increased grooming. Rolipram was the most potent compound in the three species. In guinea pigs it was less active than in rats or mice. Ro 20-1724 was approx. 15 to 30 times less potent in inducing the characteristic alterations in the various species. The alkylxanthine PDE inhibitor IBMX, 0.39 to 6.25 mg/kg, slightly stimulated the locomotor activity of mice and rats, most probably due to antagonism of central adenosine actions. IBMX, 6.25 to 25 mg/kg, caused a pattern of neurotropic effects identical to that produced by the selective cAMP PDE inhibitors, indicating the prevalence of the cAMP PDE inhibitory action over the adenosine antagonistic action at higher dosages. IBMX was approx. as potent as Ro 20-1724 in this respect. The species differences in the neurotropic responses to cAMP PDE inhibition *in vivo* presumably reflect similar differences in the extent of cAMP accumulation in brain tissue of the three species *in vitro*. Enhanced availability of brain cAMP *in vivo* in the various rodent species seems to be correlated with diverse patterns of more or less complex motor behavioural symptoms.

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Key words: Rolipram, phosphodiesterase inhibitors, locomotor activity, rectal temperature, head twitches, grooming, forepaw shaking, mouse, rat, guinea pig.

Introduction

We recently reported that the phosphodiesterase (PDE) inhibitors rolipram, Ro 20-1724 or ICI 63 197 induce a conspicuous behavioural syndrome in rats which is characterized by hypokinesia, hypothermia and head twitches associated with forepaw shaking and increased grooming (*Wachtel*, 1978; *Wachtel et al.*, 1980; *Wachtel*, 1982). These behavioural alterations were mimicked by dibutyryl adenosine cyclic 3′, 5′-monophosphate (dBcAMP) administered either intracerebroventricularly or systemically; dibutyryl guanosine cyclic 3′, 5′-monophosphate (dBcGMP) was ineffective in this respect (*Wachtel*, 1978; *Wachtel*, 1982). Since rolipram, Ro 20-1724 and ICI 63 197 have been shown to possess a high selectivity towards cAMP rather than cGMP PDE of rat brain preparations (*Schwabe et al.*, 1976; *Butt et al.*, 1979), we have suggested that the characteristic behaviour reflects the enhanced *in vivo* availability of cAMP in the brain induced by these compounds (*Wachtel*, 1978; *Wachtel et al.*, 1980; *Wachtel*, 1982). The behavioural alterations produced by rolipram, Ro 20-1724, ICI 63 197 or dBcAMP in rats are reminescent of components of the "quasimorphine withdrawal syndrome" (QMWS) described by Collier and co-workers following systemic administration of the PDE inhibitors 3-isobutyl-1-methylxanthine (IBMX), theophylline, caffeine, Ro 20-1724, and ICI 63 197 to rats (*Collier et al.*, 1981; for review). The authors concluded that these drugs act by inhibiting brain cAMP PDE, thus raising cerebral cAMP levels. These findings have been taken as evidence in favour of the hypothesis that the true morphine withdrawal syndrome is due to an increased activity of a neuronal cAMP mechanism (*Collier* and *Francis*, 1975; *Francis et al.*, 1975). In the course of studies on the PDE inhibitor-induced QMWS, *Collier* (1974) observed that the alkylxanthine PDE inhibitor theophylline produced head twitches in rats but not in mice indicating species differences in the behavioural effects of PDE inhibitors. Preliminary investigations revealed that rolipram caused neuroleptic-like effects in mice but, in contrast to rats, did not induce head twitches in this species (*Wachtel*, 1979; *Wachtel et al.*, 1979). Numerous *in vitro* studies have shown marked species differences with respect to the extent of cAMP accumulation in slices of a given brain region following various stimulatory agents (*Daly*, 1977; for review). These

differences may be explained either by differences in synthetic or in degradative factors regulating cerebral cAMP availability. In the present study evidence is presented that in three different rodent species enhancement of brain cAMP availability *in vivo* by inhibition of cAMP degradation with potent and selective cAMP PDE inhibitors manifests itself in differential behavioural syndromes.

Materials and Methods

A. Drugs and Solutions

The following drugs were used: (±)-rolipram (4-[3-cyclopentyloxy-4-methoxy-phenyl]-2-pyrrolidone, Schering AG, Berlin/Bergkamen, FRG), Ro 20-1724 (4-[3-n-butyloxy-4-methoxy-benzyl]-2-imidazolidinone, Hoffmann - La Roche, Basel, Switzerland), 3-isobutyl-1-methylxanthine (IBMX, EGA-Chemie KG, Steinheim, FRG). All compounds were suspended in isotonic saline solution containing 10% w/v Cremophor EL® (polyethoxylated castor oil, BASF, Ludwigshafen, FRG).

B. Animals and Treatment Schedules

Male NMRI mice (Department Tierzucht und -haltung, Schering AG, Berlin/Bergkamen, FRG; body weight 20—25 g), male Wistar rats (Department Tierzucht und -haltung, Schering AG, Berlin/Bergkamen, FRG; body weight 80—140 g) and Pirbright White guinea pigs (Tierfarm Bruno Buchner, Kienberg, FRG; body weight 170—330 g) were used. The animals were kept in a temperature-controlled room (22 ± 1 °C) with a 12 hours light/dark cycle. The room was light between 6 a.m. and 6 p.m. The animals received a standard diet of food pellets (Altromin®, Altrominfutterwerke, Lage, FRG) up to the time of experimentation. All compounds were administered by the intraperitoneal route in a volume of 1 ml/100 g of body weight (mice) or 0.5 ml/100 g of body weight (rats, guinea pigs) with randomized allocation of treatment. Control animals received the appropriate volume of the vehicle. All experiments were performed between 9 a.m. and 4 p.m.

C. Methods

1. Body Temperature

At 1 hour prior to experimentation, individual animals were placed into transparent plastic cages (25 × 19 × 13.5 cm). Immediately before and 60 min after drug or vehicle administration, body temperature was measured with an electric thermometer (ELLAB TE 3, Electrolaboratoriet, Copenhagen, Denmark) for 20 sec after introduction of a rectal probe (RM 6 in experiments with mice; RM 4 in experiments with rats and guinea pigs).

Table 1. *Effect of various doses of the phosphodiesterase inhibitors rolipram, Ro 20-1724 or isobutylmethylxanthine (IBMX) upon rectal temperature of mice, rats or guinea pigs*

Species		Rectal temperature (°C)					
		Vehicle	Compound	0.39	1.56	6.25	25 mg/kg
Mouse	(8)	36.2 ± 0.4	rolipram	35.9 ± 0.5	35.5 ± 0.4	35.3 ± 0.4	34.7 ± 0.4*
		36.0 ± 0.4	Ro 20-1724	35.6 ± 0.4	36.3 ± 0.4	35.7 ± 0.3	35.6 ± 0.4
		36.2 ± 0.4	IBMX	35.7 ± 0.3	36.2 ± 0.4	35.7 ± 0.2	35.1 ± 0.2*
Rat	(8)	36.8 ± 0.2	rolipram	35.9 ± 0.2*	35.6 ± 0.3**	35.5 ± 0.2**	35.2 ± 0.2**
		37.3 ± 0.1	Ro 20-1724	37.6 ± 0.1	37.4 ± 0.2	36.8 ± 0.2	35.9 ± 0.2**
		37.5 ± 0.1	IBMX	37.6 ± 0.3	37.4 ± 0.3	36.0 ± 0.3**	36.0 ± 0.4**
Guinea pig	(8)	37.6 ± 0.2	rolipram	37.2 ± 0.2	36.8 ± 0.2*	36.5 ± 0.2**	36.6 ± 0.1**
		37.4 ± 0.2	Ro 20-1724	37.0 ± 0.3	37.2 ± 0.2	37.0 ± 0.2	36.4 ± 0.3**
		37.4 ± 0.2	IBMX	37.2 ± 0.2	37.2 ± 0.2	37.4 ± 0.1	35.5 ± 0.3**

The rectal temperature of individually-housed animals was measured by means of an electric thermometer 60 min following intraperitoneal administration of the various drug dosages or of the vehicle. The values are means ± S.E.M. The number of animals per treatment group is indicated in brackets. Statistical significances of the differences between the various drug dosages and the corresponding control are shown (** $p < 0.01$; * $p < 0.05$; one-way analysis of variance followed by Dunnett test).

2. Locomotor Activity

Animals were given various doses of the test compounds or the vehicle and immediately thereafter were individually placed into circular photocell motility cages described in detail elsewhere (*Wachtel*, 1982). The number of light beam interruptions accumulated during 10-min intervals was recorded for 60 min.

3. Head Twitches

Immediately following drug or vehicle administration, individual animals were placed into transparent plastic cages (25 × 19 × 13.5 cm). After 15 min, the incidence of head twitches (short-lasting, rapid, repetitive oscillations along the longitudinal axis of the head or upper trunk with subsequent return to the resting position) was counted for 60 min by an experienced observer who was not aware of the treatment. Each laboratory assistant usually observed five animals simultaneously for a 1-hour session.

D. Statistics

Means ± S.E.M. were calculated for the various treatment groups and the statistical significances of the differences between the means of the various drug dosages and the corresponding control were determined by one-way analysis of variance in conjunction with the Dunnett test.

Results

1. Influence upon Rectal Temperature

60 min following pretreatment with the PDE inhibitors rolipram, Ro 20-1724 or IBMX in dosages ranging from 0.39 to 25 mg/kg i.p., the rectal temperature of rats and guinea pigs was lowered in a dose-related manner (Table 1). In both species rolipram was the most potent compound. Rolipram and IBMX were more effective in rats than in guinea pigs. In mice, rolipram and IBMX caused a slight, statistically significant fall of the rectal temperature only at the highest dose investigated (25 mg/kg). Ro 20-1724, 0.39 to 25 mg/kg, did not influence the thermoregulation of mice; non-toxic dosages higher than 25 mg/kg, however, induced hypothermia in this species as well (data not shown).

2. Influence upon Locomotor Activity

At dosages of 0.39 to 25 mg/kg i.p., rolipram and Ro 20-1724 dose-dependently decreased locomotor activity of mice and rats

Table 2. *Effect of various doses of the phosphodiesterase inhibitors rolipram, Ro 20-1724 or isobutylmethylxanthine (IBMX) upon locomotor activity of mice, rats or guinea pigs*

Species		Counts per 60 min					
		Vehicle	Compound	0.39	1.56	6.25	25 mg/kg
Mouse	(9)	838 ± 152	rolipram	417 ± 110**	311 ± 57**	72 ± 27**	41 ± 13**
		744 ± 108	Ro 20-1724	819 ± 299	894 ± 259	475 ± 101	95 ± 27**
		729 ± 109	IBMX	893 ± 115	1051 ± 241	737 ± 222	131 ± 28*
Rat	(8)	650 ± 63	rolipram	434 ± 16**	410 ± 47**	380 ± 37**	268 ± 62**
		535 ± 55	Ro 20-1724	523 ± 64	343 ± 34*	329 ± 22*	255 ± 72**
		472 ± 28	IBMX	556 ± 135	588 ± 66	734 ± 142	604 ± 97
Guinea pig	(7)	310 ± 46	rolipram	379 ± 86	495 ± 79	796 ± 205*	1375 ± 207**
		310 ± 46	Ro 20-1724	268 ± 52	350 ± 47	389 ± 55	771 ± 86**
		310 ± 46	IBMX	353 ± 60	415 ± 131	589 ± 207	890 ± 165**

Immediately following intraperitoneal administration of the various drug dosages or of the vehicle individual animals were placed into photocell motility cages and the counts accumulated during 60 min were recorded. The values are means ± S.E.M. The number of animals per treatment group is indicated in brackets. Statistical analysis was as in legend to Table 1.

Table 3. *Induction of head twitches by various doses of the phosphodiesterase inhibitors rolipram, Ro 20-1724 or isobutylmethylxanthine (IBMX) in rats or guinea pigs*

Species	Number of head twitches per 60 min					
	Vehicle	Compound	0.39	1.56	6.25	25 mg/kg
Rat (8)	0.5 ± 0.3	rolipram	15 ± 3**	23 ± 4**	33 ± 5**	43 ± 6**
	0.3 ± 0.2	Ro 20-1724	2 ± 1	3 ± 1	15 ± 3**	29 ± 5**
	1 ± 0.3	IBMX	1 ± 0.4	4 ± 1	29 ± 3**	42 ± 8**
Guinea pig (7)	0.1 ± 0.1	rolipram	3 ± 1	4 ± 1*	12 ± 4**	28 ± 3**
	0.1 ± 0.1	Ro 20-1724	1 ± 0.5	2 ± 1	3 ± 1	14 ± 5**
	0.1 ± 0.1	IBMX	0.1 ± 0.1	1 ± 1	4 ± 2	20 ± 4**

The occurrence of head twitches of individually-housed animals was counted from the 15th to 75th min following intraperitoneal administration of the various drug dosages or of the vehicle. The values are means ± S.E.M. The number of animals per treatment group is indicated in brackets. Statistical analysis was as in legend to Table 1.

(Table 2). IBMX caused hypokinesia in mice only at the highest dosage investigated (25 mg/kg); lower doses of IBMX (0.39 and 1.56 mg/kg) slightly stimulated locomotor activity of mice, an effect also seen throughout the entire dosage range of IBMX in rats. This tendency towards an increase of locomotor activity by IBMX in mice and rats, however, did not reach the level of statistical significance. In guinea pigs, all compounds caused a dose-related motor stimulation. Rolipram was the most potent of the three drugs to induce statistically significant alterations of the locomotor activity of mice, rats and guinea pigs.

3. Induction of Head Twitches

At dosages of 0.39 to 25 mg/kg i.p., the PDE inhibitors rolipram, Ro 20-1724 and IBMX dose-dependently induced head twitches in rats and guinea pigs (Table 3). In both species rolipram was more potent than Ro 20-1724 or IBMX; both, Ro 20-1724 and IBMX, were approx. equieffective in either species. The three compounds were more effective in rats than in guinea pigs. In both species the PDE inhibitor-induced head twitches were regularly accompanied by forepaw shaking and increased grooming; additional symptoms emerging were chewing, vocalization on touch, mild ptosis, and salivation. Qualitatively the head twitch reaction was more pronounced in guinea pigs in that it was intensified rather often to body shakes ("wet dog shakes"), an effect rarely observed in rats after rolipram or Ro 20-1724. Rolipram, Ro 20-1724 or IBMX in dosages ranging from 0.1 mg/kg i.p. up to lethal doses (100 mg/kg i.p. of IBMX; 800 mg/kg i.p. of rolipram or Ro 20-1724) did not increase the incidence of head twitches in mice above the level of vehicle-treated controls (maximally 3 head twitches per 60 min; data not shown).

Discussion

The present data demonstrate marked differences in CNS responses of mice, rats and guinea pigs to the PDE inhibitors rolipram, Ro 20-1724 and IBMX. The most pronounced species differences were seen with respect to motor behaviour (locomotor activity, maintenance behaviour) whereas thermoregulation was influenced uniformly (hypothermia). The 3-alkoxy-4-methoxyphenyl derivatives rolipram and Ro 20-1724, which preferentially inhibit cAMP PDE in brain preparations (*Schwabe et al.*, 1976; *Butt et al.*, 1979), caused hypothermia and *hypo*kinesia in mice, hypothermia, *hypo*kinesia and head twitches in rats, and hypothermia, *hyper*kinesia and head

twitches in guinea pigs. The head twitches were associated with forepaw shaking and increased grooming. These findings suggest that enhanced *in vivo* availability of cerebral cAMP induced by these PDE inhibitors in the various species manifests itself in hypothermia and in more or less complex motor behavioural symptoms being typical for a particular species. Ro 20-1724, in accordance with its less pronounced cAMP PDE inhibitory action *in vitro* (*Schwabe et al.*, 1976), was approx. 15 to 30 times less potent than rolipram.

In contrast to rolipram and Ro 20-1724, the alkylxanthine derivative IBMX slightly stimulated the locomotor activity of mice and rats (Table 2). Although this effect was not statistically significant, it merits some comments. IBMX, as opposed to rolipram and Ro 20-1724, shows only a low substrate selectivity towards brain PDE and inhibits cGMP degradation in only slightly higher concentrations (*Minneman*, 1976; *Butt et al.*, 1979). The locomotor stimulatory effect of another alkylxanthine derivative, caffeine, in mice has causally been related to an increased cerebral cGMP/cAMP ratio; this neurobiochemical finding, however, with good reason was not attributed to PDE inhibitory action of caffeine (*Sprügel et al.*, 1977). Although IBMX is a much more potent PDE inhibitor than caffeine or theophylline (*Smellie et al.*, 1979), one should realize that IBMX, in addition to the less selective PDE inhibitory action, acts as a potent adenosine receptor antagonist in brain preparations of mice (*Snyder et al.*, 1981), rats (*Prémont et al.*, 1977) and guinea pigs (*Huang et al.*, 1972). Adenosine is considered as an inhibitory modulator in the CNS (*Burnstock*, 1975; for review). Therefore, it is conceivable that the stimulatory effect of IBMX might be due to the blockade of adenosine receptors, a suggestion supported by interaction studies with various alkylxanthine PDE inhibitors and adenosine analogues on mouse locomotor activity recently published by *Snyder et al.* (1981). At dosages of IBMX beyond 6.25 mg/kg the slight locomotor stimulatory effect declined (rats) or even changed into hypokinesia (mice). Since alkylxanthines exert PDE inhibition at considerably higher concentrations than required to block adenosine effects (*Smellie et al.*, 1979), the decline of the stimulatory effect following high doses of IBMX would indicate the emerging prevalence of cAMP PDE inhibition over the competing adenosine antagonistic action, an explanatory attempt previously proposed for the biphasic action of the alkylxanthine PDE inhibitor theophylline and IBMX upon rat locomotor activity (*Wachtel et al.*, 1980; *Wachtel*, 1982). This assumption is further strengthened by the finding that at higher doses of IBMX additionally conspicuous symptoms (head twitches associated with forepaw shaking and increased grooming) emerged in

rats, which very effectively were elicited by the selective cAMP PDE inhibitor rolipram. Furthermore, the previous observation that i.p. administration of dBcAMP, which is considered to penetrate cellular membranes and to be resistant to inactivation by cyclic nucleotide PDE (*Henion et al.*, 1967; *Kuo et al.*, 1974) produced drowsiness and locomotor depression in mice (*Henion et al.*, 1967; *Weiner* and *Olson*, 1973) and sedation in rats (*Beer et al.*, 1972) and the present findings demonstrating the clearcut locomotor depressant effect of the selective cAMP PDE inhibitors rolipram and Ro 20-1724, support the assumption that enhanced availability of cerebral cAMP rather than cGMP might be responsible for the sedative properties of IBMX. Consonant with the suggestion that enhanced availability of brain cAMP is accompanied by a fall of body temperature, are recent reports demonstrating the hypothermic action of intracerebrally administered dBcAMP in mice (*Dascombe et al.*, 1980) and rats (*Dascombe* and *Parkes*, 1981). Finally, determinations of brain cyclic nucleotide levels following systemic administration of non-toxic dosages of IBMX, rolipram and Ro 20-1724 in rats (*Schneider* and *Prozesky*, 1979; *Kant et al.*, 1980) or of rolipram in mice (*Schwabe*, unpublished results) demonstrate that the compounds studied in fact are able to increase brain cAMP levels *in vivo*.

The species differences in behavioural responses to rolipram, Ro 20-1724 and IBMX reported here presumably are an *in vivo* reflexion of quantitative and qualitative differences reported for the extent of cAMP accumulation in brain tissue of the three rodent species *in vitro* (*Daly*, 1977; for review). The response of mice, rats and guinea pigs to cAMP PDE inhibition *in vivo* profoundly differed with respect to motor behavioural symptoms.

Regional analyses have revealed particularly high cAMP PDE activity in cerebral cortex, limbic system and striatum (*Weiss* and *Costa*, 1968; *Krishna et al.*, 1970), *i.e.* in brain structures which play an important role in regulation of motor behaviour. It might be speculated that the dissimilar motor behaviour induced by cAMP PDE inhibition in the three species reflects functional differences in the regulation of cAMP availability predominantly in these brain structures. Brain PDE is only one, albeit important, factor influencing the extent of accumulation of neuronal cAMP. Thus, apart from differences in basal activity, substrate affinity and pattern of PDE isoenzymes within these brain structures of the various species, differences in basal activity of adenylate cyclases, the degree of their activation by neurotransmitters or differences in the basal activity and pattern of cAMP-dependent protein kinase isoenzymes could contribute to the observed species differences. Differences in the

nature of receptors controlling cAMP formation have also to be taken into account (*Daly*, 1977; for review). Recently published data on species differences in content of endogenous peptide inhibitors of brain cAMP PDE offer further explanatory possibilities (*Collier et al.*, 1982).

In the present report the previous finding of *Collier* (1974), that the classic alkylxanthine PDE inhibitor theophylline produced head twitches in rats but not in mice, is extended to other more potent and more selective PDE inhibitors. Furthermore, this report for the first time demonstrates that the alkylxanthine PDE inhibitor IBMX and the selective cAMP PDE inhibitors rolipram and Ro 20-1724 induce head twitches in guinea pigs. Head twitches are thought to be mediated via stimulation of central 5-hydroxytryptamine (5-HT) receptors (*Jacobs*, 1976; for review). The fact that the PDE inhibitors rolipram, Ro 20-1724 or IBMX produced head twitches in rats and guinea pigs but not in mice, casts doubts upon the concept that the PDE inhibitor-induced head twitches are mediated via a 5-HT receptor mechanism. Furthermore, the PDE inhibitor-induced head twitches were associated with increased maintenance activity (grooming), an effect not seen in conjunction with head twitches evoked by 5-HT receptor stimulation. Interaction studies in rats and guinea pigs revealed that rolipram-induced head twitches were antagonized only weakly by very high doses of 5-HT antagonists, but very effectively by reasonable dosages of morphine and clonidine (*Wachtel*, 1979), a finding recently confirmed in rats by *Przegalinski et al.* (1981), indicating the involvement of opioid and adrenergic receptor mechanisms rather than serotoninergic receptor mechanisms. These findings can be taken as further evidence in favour of the hypothesis that the morphine withdrawal syndrome is accompanied by increased neuronal cAMP availability (*Collier* and *Francis*, 1975; *Francis et al.*, 1975).

In conclusion, by using the selective cAMP PDE inhibitors rolipram and Ro 20-1724, this report presents evidence that enhanced availability of brain cAMP *in vivo* manifests itself in differential behavioural syndromes in mice, rats and guinea pigs. Profound species differences were seen with respect to motor behavioural symptoms (locomotor activity, incidence of head twitches associated with forepaw shaking and increased grooming), whereas thermoregulation was influenced in the same direction (hypothermia). The species differences in behavioural responses to cAMP PDE inhibition reported here presumably reflect quantitative and qualitative differences in the extent of cAMP accumulation in brain tissue of the three rodent species *in vitro* documented in the literature (*Daly*, 1977; for review). In the present report our previous finding, that selective

cAMP PDE inhibitors like rolipram, Ro 20-1724 or ICI 63 197 produce a characteristic behavioural syndrome in rats (*Wachtel et al.*, 1980; *Wachtel*, 1982), is extended to guinea pigs and mice. Our data provide evidence for the interrelation between enhanced cerebral cAMP availability *in vivo* and brain function as is manifested in well defined behavioural alterations being typical for a particular species. Because of the potency and selectivity of the neurotropic cAMP PDE inhibitory action *in vivo*, compounds like rolipram could offer a potential contribution to studies on the functional role of brain cAMP in the neuroethology of complex mammalian behaviour patterns *e.g.* in continuation of previous attempts at correlating brain cAMP accumulation and behaviour in genetically defined inbred strains of rats (*Skolnick* and *Daly*, 1974) and mice (*Sattin*, 1975; *Stalvey et al.*, 1976).

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