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Neurotropic effects of the optical isomers of the selective adenosine cyclic 3′,5′-monophosphate phosphodiesterase inhibitor rolipram in rats in-vivo

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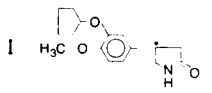
The efficacy of the selective adenosine cyclic 3′,5′-monophosphate (cAMP) phosphodiesterase (PDE) inhibitor (±)-rolipram and its optical isomers (0·006 to 25 mg kg^{-1}) in inducing characteristic behavioural changes like hypothermia, hypoactivity, forepaw shaking, grooming and head twitches in rats has been examined. (+)-Rolipram was found some 15 times less potent than the racemate suggesting a stereoselective interaction with a rat brain cAMP phosphodiesterase isoenzyme. Following their intracerebral administration, the stereoisomers also demonstrated their unusual potency ratio. These findings suggested that (+)-rolipram is a less potent neurotropic PDE inhibitor in-vivo than its (−)-enantiomer.

Rolipram, 4-(3-cyclopentyloxy-4-methoxy-phenyl)-2-pyrrolidone; Ro 20-1724 (I) and ICI 63 197 (2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-S-triazolo [1,5a]pyrimidine) are novel neurotropic phosphodiesterase (PDE) inhibitors which have the ability to inhibit, preferentially, adenosine cyclic 3′,5′-monophosphate (cAMP) phosphodiesterase in rat brain preparations in-vitro (Sheppard et al 1971; Schwabe et al 1976; Butt et al 1979). As their potency in inhibiting in-vitro rat brain cAMP PDE is related to their efficacy in inducing a behavioural syndrome in rats, characterized by hypoactivity, head twitches, forepaw shaking, grooming and hypothermia, it has been suggested that the effects mimicked by dibutyryl cAMP but not dibutyryl cGMP, reflected the enhanced availability of cerebral cAMP induced by these PDE inhibitors in-vivo (Wachtel 1978, 1982; Wachtel et al 1980b). This assumption was confirmed by determinations of rat brain cAMP levels following the systemic administration of the inhibitors (Arbuthnott et al 1974; Schneider & Prozesky 1979; Kant et al 1980). Analysis of behavioural responses would thus seem to be a suitable procedure for recognizing neurotropic compounds with selective cAMP phosphodiesterase inhibitory activity and for characterizing their potency. This was demonstrated for (±)-rolipram and confirmed by Schwabe et al 1976.



I Rolipram (ZK 62711)

* Asymmetric carbon atom.

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We have compared the behavioural effects of (±)-rolipram and its (−)- and (+)-isomers upon body temperature, locomotor activity and the incidence of head twitches, forepaw shaking and grooming in rats.

MATERIALS AND METHODS

Drugs and solutions

Drugs used were: (±)-Rolipram, (−)-rolipram and (+)-rolipram (prepared by Dr R. Schmiechen, Department of Medicinal Chemistry, Schering AG, Berlin, FRG) and lisuride hydrogenmaleate (LHM) (Spofa, Prague, Czechoslovakia). The compounds were suspended in isotonic saline (0·9% NaCl) solution containing 10% w/v Cremophore EL (polyethoxylated castor oil, BASF, Ludwigshafen, FRG). Nembutal (sodium pentobarbitone form for veterinary use, 60 mg ml^{-1}; Abbott, North Chicago, USA) was used for anaesthesia.

Animals and treatment schedules

Male Wistar rats (Department Tierzucht und -haltung, Schering AG, Berlin, FRG) ca 100 g and 200 g for systemic and intracerebral treatment respectively, were kept in a room at 22 ± 1 °C with a 12 h light/dark cycle (light between 6 am and 6 pm). The animals received a standard diet (Altromin, Altromin Spezialfutterwerke GmbH, Lage, FRG) with free access to water. The compounds were administered intraperitoneally (i.p.) in a volume of 0·5 ml per 100 g weight or intracerebrally in a

volume of 1 μl into the nucleus accumbens on each side with randomized allocation of treatment. The dosage of lisuride hydrogenmaleate refers to the base. Control animals received a corresponding volume of vehicle. All experiments were performed between 9 am and 4 pm.

Surgery and intracerebral injection

This procedure was as described by Wachtel & Andén (1978). Guide cannulae aimed at the nucleus accumbens were implanted bilaterally by means of a stereotaxic instrument (David Kopf Instruments, Tujunga, USA) in pentobarbitone-anaesthetized animals. 48–72 h post-operatively drugs were administered to conscious animals by introducing injection cannulae bilaterally to the nucleus accumbens. The coordinates of the tips of the injection cannulae were determined from the atlas of König & Klippel (1963): A 9·4, L 1·0, V −0·9. Each animal was used once only. After the test, the brains were removed and placed in 10% w/v formalin for at least 24 h. The positions of the injection cannulae were examined by inspection of the tracts of sectioned brains, where necessary, under a microscope.

Locomotor activity

Locomotor activity after i.p. treatment was measured using circular photocell activity cages (Wachtel 1982a). For interaction studies following intra-accumbens injections M/P 40 Fc Electron Motility Meters (Motron Products, Stockholm, Sweden) were used (Modigh 1972). Every interruption of the photocell beam was recorded as one count. Immediately following drug or vehicle administration, individual rats were placed in a motility cage and the counts accumulated over 10 min intervals were recorded for 60 min.

Head twitches

Immediately after drug or vehicle administration, individual rats were placed into transparent plastic cages (25 × 19 × 13·5 cm). 15 min later the number of head twitches (shortlasting rapid repetitive rotary oscillations of the head) was counted over 60 min by an experienced observer unaware of the animal's treatment.

Forepaw shaking and grooming

Following drug or vehicle administration, individual rats were placed into cylindrical transparent plastic cages (diameter 30 cm, height 40 cm). 15 min later the incidence of forepaw shaking, grooming or head twitches was counted for 60 min by an experienced observer unaware of the animal's treatment. The following criteria were used, forepaw shaking: rapid repetitive horizontal shaking of the forepaws with the animal standing on its hindlimbs, and grooming: a complete sequence of movements consisting of washing the snout and face, brushing the fur of the head and ears, and flanks with the forelimbs, accompanied by licking the flanks.

Body temperature

One h before testing, individual animals were placed into transparent plastic cages (25 × 19 × 13·5 cm). Immediately before and 30 min after drug or vehicle administration body temperture was measured with an electric thermometer (ELLAB TE 3, Elektrolaboratoriet, Copenhagen, Denmark) for 20 s after introduction of a rectal probe (RM 4). As introduction of a rectal probe produces a sustained elevation of core temperature in rats (Poole & Stephenson 1977) a drug-induced decrease of body temperature could be even more pronounced than is reflected by the rectal thermometer technique used in this study.

Statistics

Means ± s.e.m. were calculated and the statistical significances of the differences between the means of the various drug doses and the control were determined by one-way analysis of variance in conjunction with the Tukey test.

RESULTS

Locomotor activity and head twitches

(±)-Rolipram and its enantiomers, 0·025 to 6·25 mg kg^{-1}, dose-dependently inhibited locomotor activity and induced head twitches (Fig. 1 and Fig. 2 respectively). After (±)-rolipram or (−)-rolipram, these effects became evident at the lowest dose (0·025 mg kg^{-1}) and were statistically significant at ≥0·39 mg kg^{-1}. (+)-Rolipram was ca 15 to 20 times less potent than the (−)-isomer or (±)-rolipram. (−)-Rolipram and the racemate were almost equally effective.

The influence of equal doses (6·25 μg per side) of (±)-rolipram and its optical isomers administered into the nucleus accumbens on the hyperactivity produced by intra-accumbens LHM (6·25 μg per side) is shown in Table 1. Co-administration of (±)-rolipram or (−)-rolipram with LHM resulted in a potentiation of the dopamine agonist-induced hyperactivity to about the same extent, whereas the (+)-isomer was ineffective in this respect.

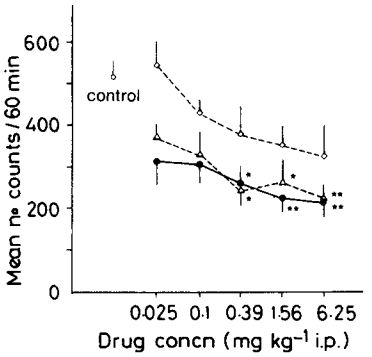

FIG. 1. Locomotor activity of rats after i.p. treatment with (±)-rolipram or of the optical isomers. (●) (±)-rolipram; (△) (−)-rolipram; (◇) (+)-rolipram. Statistical significances of the differences between the drug doses and the control are (**$P < 0.01$; *$P < 0.05$; one-way analysis of variance followed by Tukey test).

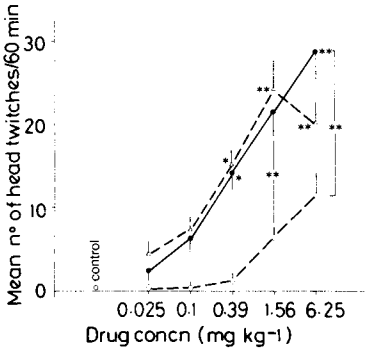

FIG. 2. Head twitches in rats after i.p. treatment with (±)-rolipram or the optical isomers. Statistical analysis and key as in Fig. 1.

Forepaw shaking and grooming

The occurrence of head twitches was associated with forepaw shaking and grooming as was demonstrated for a selected dose (1·56 mg kg^{-1}) of (±)-rolipram and its enantiomers (Fig. 3). (+)-Rolipram was significantly less potent than the (−)-isomer or (±)-rolipram in inducing head twitches, forepaw shaking and grooming whereas (−)-rolipram and the racemate were about equally effective. The generally higher frequency of head twitches in this experiment (and also in that shown in Table 2) compared with that shown in Fig. 2 might be explained by the smaller number of animals being observed per test session (2 instead of 5) presumably allowing a more accurate perception of a shortlived behavioural phenomenon.

Table 1. Influence of intra-accumbens lisuride hydrogen-maleate (LHM) alone or in combination with (±)-rolipram or its optical isomers on rat locomotor activity.

Treatment	Counts/60 min
LHM	2343 ± 396
LHM plus (±)-rolipram	4336 ± 619[c]
LHM plus (−)-rolipram	4791 ± 641[ad]
LHM plus (+)-rolipram	1898 ± 519

Immediately following the injection of LHM 6·25 μg or of a mixture of LHM 6·25 μg plus 6·25 μg of (±)-rolipram, (−)-rolipram or (+)-rolipram into the nucleus accumbens on each side, individual rats were placed into photocell motility cages and the counts accumulated during 60 min were recorded. The values are means ± s.e.m. of 6 experiments. Statistical significances of the differences between the various treatments are shown (a: $P < 0.05$ vs LHM alone; c: $P < 0.05$ vs LHM plus (+)-rolipram; d: $P < 0.01$ vs LHM plus (+)-rolipram; one-way analysis of variance followed by Tukey test).

Body temperature

(±)-Rolipram and its enantiomers, 0·006 to 25 mg kg^{-1}, caused a dose-related fall in rectal temperature (Fig. 4). After (±)-rolipram, the effect became evident at 0·1 mg kg^{-1} and was statistically significant at ≥0·39 mg kg^{-1}. (−)-Rolipram was slightly more potent than the racemate; it caused statistically significant hypothermia at doses ≥0·1 mg kg^{-1}. (+)-Rolipram was about 15 to 20 times less potent than the (−)-isomer or (±)-rolipram. The hypothermia did not exceed 34 °C. This maximum effect was reached with 0·39 mg kg^{-1} of (−)-rolipram and 1·56 mg kg^{-1} of (±)- rolipram, remaining at this level

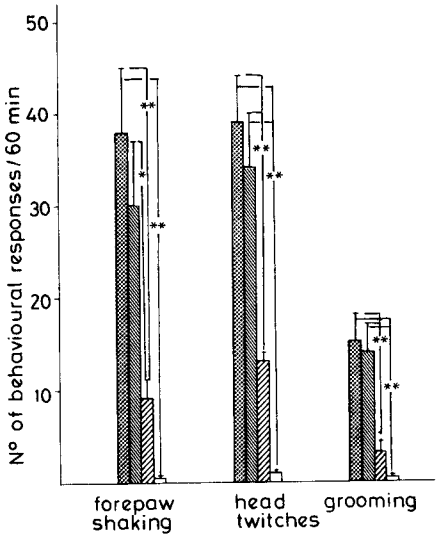

FIG. 3. Forepaw shaking, head twitches and grooming in rats following 1·56 mg kg^{-1} i.p. of (±)-rolipram or of the optical isomers. Stippled column (±)-rolipram; open column control; close hatched column (−)-rolipram; wide hatched column (+)-rolipram. Statistical analysis as in Fig. 1.

Table 2. Locomotor activity, rectal temperature and the occurrence of forepaw shaking, head twitches and grooming in rats following intraperitoneal administration of (±)-rolipram (1.56 mg kg^{-1}), (−)-rolipram (0·78 mg kg^{-1}), (+)-rolipram (0·78 mg kg^{-1}), or of a mixture of equal parts (0·78 mg kg^{-1}) of (−)- and (+)-rolipram.

Treatment	Locomotor activity (counts/60 min) n = 7	Rectal temperature (°C) n = 8	Forepaw shaking (number of behavioural responses/60 min) n = 8	Head twitches n = 8	Grooming n = 8
Vehicle	456 ± 61	36·8 ± 0·2	2 ± 1	1 ± 0·3	2 ± 1
(±)-Rolipram	186 ± 21[b]	34·3 ± 0·3[bd]	28 ± 7[ac]	29 ± 8[ac]	17 ± 4[ac]
(−)-Rolipram	143 ± 13[b]	34·4 ± 0·2[bd]	27 ± 6[ac]	26 ± 9[ac]	24 ± 5[bd]
(+)-Rolipram	330 ± 75	35·9 ± 0·1[a]	3 ± 1	3 ± 1	3 ± 1
Mixture	171 ± 24[b]	34·7 ± 0·2[bd]	40 ± 10[bd]	31 ± 6[bc]	18 ± 3[ac]

The values are means ± s.e.m. Statistical significances of the differences between the various treatments are shown (a: P <0·05 vs vehicle; b: P <0·01 vs vehicle; c: P <0·05 vs (+)-rolipram; d: P <0·01 vs (+)-rolipram; one-way analysis of variance followed by Tukey test).

up to the highest dose tested (25 mg kg^{-1}). After (+)-rolipram, maximum hypothermia was obtained at 25 mg kg^{-1}.

(±)-Rolipram compared with a mixture of equal parts of (+)- and (−)-rolipram or the proportionate amount of the individual isomers

The effects of (±)-rolipram 1·56 mg kg^{-1}, (−)-rolipram, (+)-rolipram or of a mixture of equal parts (0·78 mg kg^{-1}) of the individual isomers on rat behaviour are shown in Table 2. In all cases, there was no statistically significant difference in the potency between (±)-rolipram, the mixture of the individual isomers and the (−)-isomer. The (+)-isomer was almost ineffective in producing the various symptoms.

DISCUSSION

These results confirm and extend previous findings in rats (Watchel 1978), indicating differences in the potency of the optical isomers of rolipram in inducing a behavioural effects characteristic of selective cAMP phosphodiesterase inhibitors (Wachtel 1978, 1982; Wachtel et al 1980(b). (−)-Rolipram was found to be approximately 15 times more potent than (+)-rolipram. Similar results were obtained in mice with respect to antagonism of reserpine-induced hypothermia (Askew 1963) and potentiation of yohimbine lethality (Quinton 1963), two classic test models for prediction of clinical antidepressant activity (Wachtel 1983). As differences in water solubility (0·02% w/v for (±)-rolipram and (−)-rolipram, 0·04% w/v for (+)-rolipram; R. Schmiechen, personal communication) or absorption had no effect on differences in the potency of the isomers (+)-rolipram was clearly shown to be less effective than the (−)-isomer or (±)-rolipram in potentiating the locomotor stimulation produced by intra-accumbens injection of the dopamine agonist LHM (Wachtel & Schlangen 1979), whereas (−)-rolipram and (±)-rolipram were almost equally effective. As the potentiating effect of (±)-rolipram on LHM-induced hyperactivity is prevented by pretreatment with the dopamine antagonist haloperidol (Wachtel et al 1980a), it is suggested that the enhancement of postsynaptic dopaminergic transmission initiated by stimulation of a dopamine receptor-coupled adenylate cyclase is brought about by an inhibition of cAMP degradation due to the phosphodiesterase inhibitory property of rolipram. The previous finding (Wachtel et al 1980a) that the potentiating effect was also present in rats depleted from biogenic amines by pretreatment with reserpine and α-methyl-*p*-tyrosine, excludes the involvement of presynaptic mechanisms. Intra-accumbens rolipram did not stimulate, but slightly inhibited locomotor activity of rats (Wachtel et al 1980a). These findings indicate that the neurotropic effects of (±)-rolipram, at doses up to 1·56 mg kg^{-1}, are attributable almost exclusively to the (−)-isomer, supporting the assumption

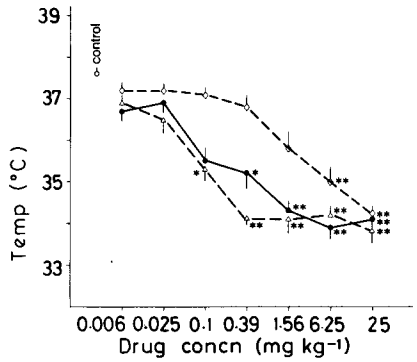

FIG. 4. Rectal temperature of rats following i.p. doses of (±)-rolipram or of the optical isomers. Statistical analysis and key as in Fig. 1.

of a highly stereoselective interaction of rolipram with a brain cAMP phosphodiesterase isoenzyme as suggested in previous work (Wachtel 1978, 1982; Wachtel et al 1980b). This is further supported by the recent finding of a close association of high affinity binding sites for rolipram and inhibition of an isolated soluble phosphodiesterase by rolipram in rat brain (Schneider 1982). Steric influences upon the inhibition of cAMP phosphodiesterase in-vitro have previously been reported for the optical isomers of RO 7-2956 (4-(3,4-dimethoxybenzyl)-2-imidazolidinone), an imidazolidinone phosphodiesterase inhibitor closely related to rolipram showing, however, the expected potency ratio with the (−)-isomer being twice, and the (+)-isomer being half, that of the racemate (Sheppard & Wiggan 1971). When equal doses of (±)-rolipram and the stereoisomers were compared (Fig. 3), no differences between the potencies of the (−)-isomer and the racemate was seen. If the (+)-isomer is less active, then the (−)-isomer should be more potent than (±)-rolipram. Failure to demonstrate such a difference is due to the high variance which, with the relatively small number of animals employed, prevented a clear differentiation of the (−)-isomer from the racemate.

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