Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 2, pp. 211-217, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00296-7

Discriminative Stimulus Properties of the Stereoisomers of the Phosphodiesterase Inhibitor Rolipram

HERBERT H. SCHNEIDER,*[1] MOTONORI YAMAGUCHI,† JOHN S. ANDREWS‡ AND DAVID N. STEPHENS*

*Research Laboratories, Schering AG, 13342 Berlin, Germany
†Nihon Schering K.K., Osaka 532, Japan
‡Organon International BV, 5340 BH Oss, The Netherlands

Received 4 May 1994

SCHNEIDER, H. H., M. YAMAGUCHI, J. S. ANDREWS AND D. N. STEPHENS. *Discriminative stimulus properties of the stereoisomers of the phosphodiesterase inhibitor rolipram.* PHARMACOL BIOCHEM BEHAV **50**(2) 211-217, 1995.—The discriminative stimulus properties of the specific type IV phosphodiesterase inhibitor, rolipram, and its two stereoisomers were assessed using standard two-lever drug discrimination procedures in which responding on the appropriate lever was reinforced on a FR10 schedule. In three separate drug cues based on training rats to discriminate the racemate (0.2 mg/kg, IP), the (−)-isomer (0.1 mg/kg), or the (+)-isomer (2 mg/kg) from vehicle, all forms substituted for one another, differing only in potency. In keeping with published reports, the (−)-isomer was the more potent form, the (+)-isomer being approximately 10 times less potent. Several phosphodiesterase (PDE) inhibitors were found to substitute for the racemate cue, their potencies in the behavioural measure correlating with their potency in displacing [^{3}H]rolipram from its forebrain binding sites in vivo (r = 0.95), suggesting that the discriminative stimulus depends on an action of the drug upon this site. Because rolipram has been reported to possess antidepressant activity, the ability of the tricyclic antidepressant imipramine to substitute for rolipram was investigated; doses of 10 and 20 mg/kg did not substitute. Amphetamine (0.156–1.25 mg/kg) also was inactive. Lisuride gave rise to drug-appropriate responding in 50% of rats only at a dose of 0.078 mg/kg, which severely disrupted responding. It is concluded that the rolipram discriminative stimulus is dependent on the selective PDE inhibititory activity of the drug, and that it does not constitute a cue based on the antidepressant property of rolipram.

Drug discrimination Rolipram PDE inhibitors Rats In vivo binding

ROLIPRAM is a specific inhibitor of the cAMP type IV phosphodiesterase isoenzyme (cAMP PDE) (3). One important role of this enzyme is in terminating the signal provided by the second messenger cAMP following its triggering by G-proteins sensitive to the activation of β-adrenergic receptors by noradrenaline, so that rolipram can be expected to increase the signal induced by activation of the brain's adrenergic system (9,18). Consistent with an upregulation of adrenergic transmission, rolipram has been shown to be active in several animal tests predictive of antidepressant activity (12,21), and an antidepressant action has been confirmed in the clinic (2,4,5).

In in vitro experiments, a specific [^{3}H]rolipram binding site has been identified in the brain (17), and in vitro autoradiographic studies have suggested that the highest density of rolipram binding sites occurs in the subiculum (8). This binding site appears to be associated with rolipram's PDE inhibitory activity (16). Subsequently, a correlation between the ability of several agents to inhibit [^{3}H]rolipram binding to forebrain structures in vivo and their behavioural effects in neuropharmacological tests was reported (14).

Drug discrimination procedures have been widely used to identify specific stimulus properties of drugs acting on the central nervous system. In general, antidepressants give rise to weak internal stimuli, and are difficult to establish as discriminative stimuli in drug discrimination procedures (1). In contrast, rolipram forms a potent discriminative stimulus in rats

[1] Requests for reprints should be addressed to Dr. H. H. Schneider, Department of Neuropsychopharmacology, Schering AG, 13342 Berlin, Germany.

(13,24). The discriminative stimulus engendered by rolipram is apparently related to rolipram's PDE inhibitory effects because, of a number of agents studied, only other PDE inhibitors substituted for rolipram. The action of rolipram is stereospecific, the (−)-isomer being approximately 8–15 times more effective in several neuropharmacological tests (12,20) and in increasing cAMP levels in rat brain (15). The interoceptive cue also appears to be stereoselective in nature: (−)-rolipram substituted more readily in a racemate-based cue than the (+)-form (13,24).

A number of publications have studied differences in the pharmacological properties of stereoisomers of optically active compounds using drug discrimination procedures [e.g., (6)], and these have been useful in identifying potential differences between the optically active forms. For this reason, it was thought useful to compare the discriminative stimuli provided by (+)- and (−)-isomers, respectively. In the present experiment, separate groups of rats were trained to discriminate the racemate, (−)- or (+)-isomer of rolipram from saline to compare and confirm the potencies in cross-generalisation experiments, and to determine their relative importance to the discriminative properties of racemic rolipram. In addition, a number of substances were tested in the racemate cue and the results correlated with their ability to displace [^{3}H]rolipram from forebrain structures in vivo.

METHOD

Animals

Male Wistar rats (Schering AG, Berlin), weighing 250 g at the beginning of the experiment, were individually housed under a 12L : 12D cycle. They were subjected to food deprivation but had free access to water; they were trained in eight operant chambers (Coulbourn Instruments Inc., Lehigh Valley, PA) fitted with house and cue lights, two levers, and a centrally located food magazine. Reinforcement scheduling and data collection were controlled by an IBM PC connected to a MedLab Associates interface (MedLab Associates Inc., East Fairfield, VT) running OPN software (19).

Training

The rats were initially shaped to press either lever on an FR1 (one pellet = one response) schedule, which was increased to FR10. Initially only one lever was reinforced per session, and this alternated from session to session. Discrimination training then began with (±)-rolipram (0.2 mg/kg), (−)-rolipram (0.1 mg/kg), or (+)-rolipram (2 mg/kg) in separate groups of eight rats. For half the rats in each group the left lever was nominated as drug lever, and for the other half the right lever was the saline lever. Rats treated with drug or saline received reward when they accumulated 10 responses (FR10) on the appropriate lever. After approximately 15 sessions the daily sequence of drug and saline treatments was randomised. For these sessions the following measures were taken: choice (first lever on which 10 responses were made), number of responses on incorrect lever before the rat pressed the correct lever 10 times, total number of responses in the 15-min session. Drug discrimination testing began when the animal had completed 10 successive sessions with the correct choice of lever (a correct choice being four or less responses on the incorrect lever before completion of the FR10 on the correct lever). In generalisation tests substances were administered IP 15 min before the session in place of the training drug. Each dose of each drug was tested in a group of eight animals. Tests were carried out twice weekly; the other 3 days were training sessions. Test days were preceded by a vehicle training day. If in one of these training sessions the wrong lever was selected, animals were given extra training sessions before reuse.

Data Analysis

During tests, the lever on which the rats first completed 10 responses was taken as the selected lever for that test session and further responding was rewarded only on that lever. The number of rats completing a selection (i.e., 10 responses on one of the two levers) was used to calculate ED_{50} values (i.e., the dose that occasioned drug-appropriate responding in 50% of the rats) initially by the method of Litchfield and Wilcoxon (10) and, when the data were inappropriate for such an analysis, by graphical approximation. Because there were only minor differences between these two evaluation methods, in the Results section only graphical estimations are reported. As a measure of sedation, the lever pressing rate, in percent of the preceding vehicle treatment, was calculated.

Drugs and Administration

All substances were dissolved in 10% Cremophor EL (CEL; BASF, Germany) in saline and applied in 1 ml/kg body weight IP 15 min before the test session. In addition to the racemate, (±)-rolipram and its stereoisomers (11), the following substances were tested for substitution in the racemate cue. Specific cAMP PDE inhibitors: ICI 63197 [2-amino-6-methyl-5-oxo-4-*n*-propyl-4,5-dihydro-*s*-triazolo(1,5a)pyrimidine; ICI, UK], TVX 2706 [3-ethyl-1-(3-nitrophenyl)-2.4-(1*H*, 3*H*)-chinazolindione; Tropon-Werke, Germany]. Nonspecific PDE inhibitor: SQ 20009 (1-ethyl-4-isopropylidenhydrazino-1*H*-pyrazolo(3,4-b)pyridine-5-carboxylic acid ethyl ester; Squibb, USA]. Non-PDE inhibitors: clobazam (Hoechst AG, Germany), lisuride (Schering AG), (+)-amphetamine (E. Merck, Germany), and imipramine (Ciba-Geigy, Switzerland). In addition, several structural analogues of rolipram were tested, ZK 47941 [4-(3,4-dimethoxyphenyl)-2-pyrrolidone], ZK 111524 [5-(4-methoxy-3-*n*-propoxy-phenyl)-5-methyl-2-oxazolidinone], and ZK 111521 [5-(3-ethoxy-4-methoxyphenyl)-5-methyl-2-oxazolidinone].

In Vivo Binding Studies

Binding studies were performed as described previously (14). Briefly, in vivo binding of [^{3}H]rolipram was determined 30 min post-IP application of drug and 2 min following the IV injection of [^{3}H]rolipram (540 kBq in 0.5 ml; 799 GBq/mmol). This schedule corresponds to the time point at the end of the drug discrimination sessions. Dissected forebrains were homogenised in 30 ml ice-cold buffer (25 m*M* sodium phosphate, pH 7.4); the homogenates were then filtered through Whatman GF/B glass fibre discs and washed three times with 3 ml of cold buffer. The influence of the compounds on [^{3}H]rolipram binding was determined using three to four doses for each compound, and four to five animals per dose (14). Basal binding and nonspecific binding were determined using vehicle or (−)-rolipram, 3 mg/kg, respectively.

RESULTS

The racemate and the two stereoisomers of rolipram all served as potent discriminative stimuli. Training to a criterion of 10 successive correct discrimination sessions required 23 sessions for the racemate, 13 sessions for the (+)-isomer, and

14 sessions for the (−)-isomer. Figure 1 shows the acquisition curves for the three cues.

Figure 2 illustrates the generalisation curves for several doses of all three training drugs in the respective cues. Figure 2A shows that in the racemate-trained animals, all three drugs substituted for the racemate in a dose-dependent fashion, and that the (−)-isomer was slightly but not significantly more potent than the racemate itself; the (+)-isomer was approximately 10 times less potent than the racemate.

In rats trained to discriminate the more active (−)-isomer (Fig. 2B), the racemate and the (−)-isomer were equally potent, (+)-rolipram being about 15 times less potent. Lastly, in rats trained to discriminate the less active (+)-isomer from vehicle (Fig. 2C), again the racemate and the (−)-isomer were approximately equipotent whereas the (+)-isomer itself was about 10 times less potent than the racemate. Table 1 summarises the drug discrimination data of the racemate and isomers in the three cues.

Table 2 shows the ED_{50} values for all substances tested for their ability to generalise to rolipram using animals trained to discriminate the racemate from saline. The most potent of these were the rolipram analogues ZK 111521 and ZK 111524, which were as potent as rolipram itself. A further analogue, ZK 47941, also substituted in this discrimination. The specific cAMP PDE inhibitor ICI 63197 was the most potent of the nonpyrrolidone-like structures. Of the other compounds with specific PDE IV inhibitory activity and displacing rolipram from its binding sites, TVX 2706 generalised to maximally 40% at the two doses tested and clobazam substituted at high doses (ED_{50} 9 mg/kg). The nonspecific PDE inhibitor, SQ 20009, gave rise to only 40% generalisation at doses up to 5 mg/kg. Amphetamine and imipramine did not induce drug-appropriate responding. Fifty percent of the lisuride-treated rats that completed a selection discriminated the drug-appropriate lever from the saline lever at a dose of 0.078 mg/kg; however, responding was severely depressed, and higher doses could not be tested.

Table 2 also shows the ED_{50} values for these substances in displacing [^{3}H]rolipram in vivo from its forebrain binding sites. Between the two sets of logarithmically transformed ED_{50}s the Pearson correlation for the eight drugs with completed data sets resulted in a coefficient of $r = 0.95, p < 0.01$ (Fig. 3).

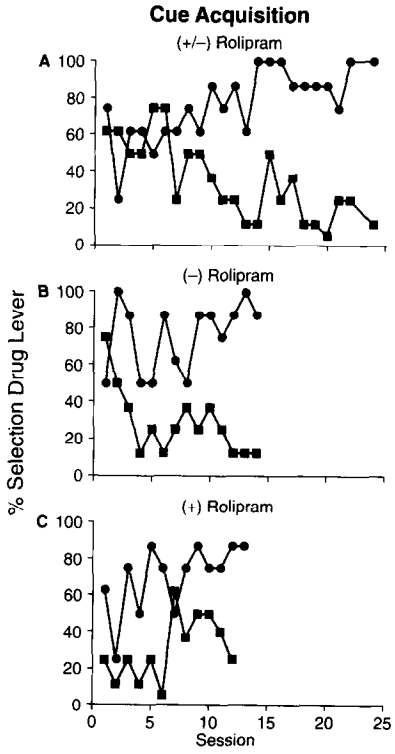

FIG. 1. Formation of drug discrimination stimulus of the respective rolipram stereoisomer through the series of training sessions. Training doses were (A) 0.2 mg/kg (±)-rolipram; (B) 0.1 mg/kg (−)-rolipram; (C) 2 mg/kg (+)-rolipram in groups of eight rats. (●) Drug, (■) saline.

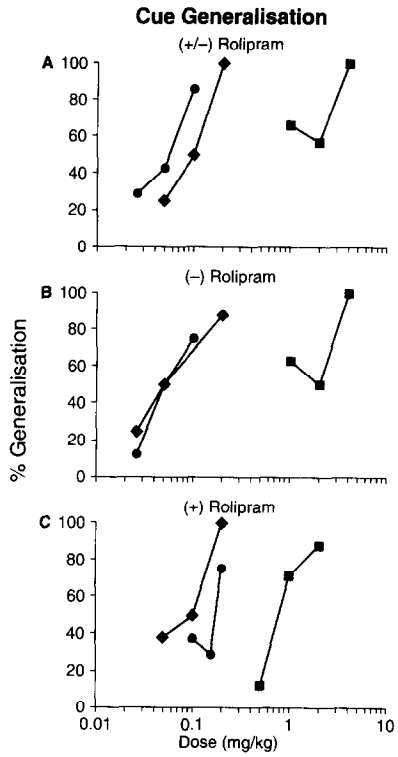

FIG. 2. Generalisation of the stereoisomers of rolipram [(♦) (±)-rolipram; (●) (−)-rolipram; (■) (+)-rolipram] given IP 15 min prior to testing to rats trained to discriminate (A) (±)-rolipram; (B) (−)-rolipram; (C) (+)-rolipram from saline.

DISCUSSION

Rolipram and its two stereoisomers can serve as effective discriminative stimuli. Each of these substances can substitute for the other and, apart from potency, behave similarly in each of the cues. The relative potency of all three drugs in all three cues was very similar, suggesting that all three discriminative stimuli are based on the same pharmacological action and that the stereoisomers do not possess further actions that contribute to the internal stimulus. The differing training doses for the cues were chosen according to the different pharmacological potencies of the enantiomers (14), so that the data obtained in the drug discrimination experiments parallel those seen in other neuropharmacological and biochemical tests in which the difference between the (−)-form and the racemate

TABLE 1

GENERALISATION TO THE DISCRIMINATIVE STIMULUS INDUCED BY THE RACEMATE, (±)-ROLIPRAM (0.2 mg/kg) AND THE (−)-ISOMER (0.1 mg/kg) AND (+)-ISOMER (2.0 mg/kg) OF ROLIPRAM

Substance	Dose (mg/kg)	N	$\frac{n - DL^*}{n_T}$	Selection Drug Lever (%)	Control Lever Press Rate (%)	ED_{50} (mg/kg)
(±)-Rolipram Cue						
(±)-Rolipram	0.05	8	2/8	25	86	0.085
	0.1	8	4/8	50	83	
	0.2	8	7/7	100	38	
(−)-Rolipram	0.025	7	2/7	29	93	0.06
	0.05	7	3/7	43	91	
	0.1	7	6/7	86	60	
(+)-Rolipram	1	8	4/7	67	53	1.0
	2	7	4/7	57	48	
	4	8	4/4	100	3	
(−)-Rolipram Cue						
(±)-Rolipram	0.025	8	2/8	25	92	0.05
	0.05	8	4/8	50	75	
	0.2	8	7/8	88	47	
(−)-Rolipram	0.025	8	1/8	13	93	0.05
	0.05	8	4/8	50	72	
	0.1	8	6/8	75	50	
(+)-Rolipram	1	8	5/8	63	62	1.5
	2	8	4/8	50	98	
	4	8	8/8	100	37	
(+)-Rolipram Cue						
(±)-Rolipram	0.05	8	3/8	38	91	0.08
	0.1	8	4/8	50	74	
	0.2	8	8/8	100	49	
(−)-Rolipram	0.1	8	3/8	38	88	0.15
	0.15	8	2/7	29	52	
	0.2	8	3/4	75	3	
(+)-Rolipram	0.5	8	1/8	13	90	0.8
	1	8	5/7	71	52	
	2	8	7/8	88	41	

*Number of rats selecting the drug lever divided by the total number of rats completing a selection.

are slight, suggesting that the effects of the racemate are mainly due to the presence of the (−)-form.

The results also show that other PDE inhibitors can effectively substitute for the racemate, and that specific PDE inhibitors appear to generalise more readily than nonspecific PDE inhibitors in the cue. Non-PDE inhibitors such as amphetamine or imipramine did not substitute for rolipram, suggesting that the cue is specific for this class of compounds. Clobazam's ability to generalise in the rolipram cue is not surprising in view of its affinity to the [^{3}H]rolipram binding site in vitro (Schneider, unpublished findings). These results confirm and extend previous studies using racemic rolipram as a discriminative stimulus (13,24). It has been reported that the behavioural effects of rolipram are mimicked by the administration of dibutyryl cAMP (20), and that rolipram raises the level of cAMP in the brain (15). For these reasons, it has been assumed that the behavioural effects of rolipram are due to an increased level of cAMP following rolipram (20). The correlation between in vivo binding potency to the rolipram-sensitive site in rat brain and substitution in the cue is suggestive that the discriminative stimulus, too, is related to rolipram's ability to increase central cAMP levels.

PDE inhibitors that bind to the rolipram-sensitive site, such as ICI 63 197, RO 20-1724, TVX 2706, and IBMX (14), also generalise to the discriminative stimulus [this study, (13,24)]. Of the specific PDE inhibitors competing with rolipram at its binding site, only CP 76,593 has been tested for antidepressant effectiveness in the clinic, with positive results [cited in (12)]. The nonspecific PDE inhibitors theophylline and caffeine, which substitute for the rolipram cue only at high doses (24), also possess potent peripheral pharmacological activities. These would preclude them from being applied in the clinic at high enough doses to occupy central rolipram binding sites to a sufficient extent.

Nevertheless, the rolipram cue appears to be based on its effects on a particular PDE system and not on its antidepres-

TABLE 2

GENERALISATION TO THE DISCRIMINATIVE STIMULUS INDUCED BY 0.2 mg/kg (±)-ROLIPRAM IN THE RAT, AND IN VIVO BINDING TO THE [^{3}H]ROLIPRAM BINDING SITE OF RAT FOREBRAIN

Substance	Dose (mg/kg)	N	$\frac{n - DL^*}{n_T}$	Selection Drug Lever (%)	Control Lever Press Rate (%)	ED_{50} Cue (mg/kg)	ED_{50} In Vivo Binding (mg/kg)
(±)-Rolipram						0.085†	0.09
(−)-Rolipram						0.06†	0.03
(+)-Rolipram						1.0†	0.48
ICI 63197	0.05	7	3/7	43	85	0.055	0.10
	0.1	7	5/7	71	71		
	0.2	7	6/7	88	70		
TVX 2706	0.2	5	2/5	40	70	> 0.78	0.46
	0.78	5	2/5	40	82		
Clobazam	5	7	1/7	14	99	9	43
	8	8	2/8	25	95		
	10	8	6/8	75	81		
	15	8	5/8	63	75		
	20	8	2/3	(67)‡	29		
ZK 47941	0.313	8	0/8	0	88	0.8	0.87
	0.625	8	3/8	38	90		
	1.25	8	5/6	83	35		
ZK 111521	0.01	6	2/6	33	115	0.023	0.05
	0.039	5	3/5	60	102		
	0.156	5	2/2	(100)	10		
ZK 111524	0.039	8	1/8	13	94	0.065	0.08
	0.055	8	2/8	25	100		
	0.078	8	6/8	75	83		
	0.156	8	5/6	83	43		
SQ 20009	1.25	7	0/7	0	99	>5	n.d.
	5	8	2/6	33	52		
(+)-Amphetamine	0.156	7	0/7	0	105	> 1.25	n.d.
	0.625	8	2/8	25	82		
	0.8	8	0/8	0	52		
	1.25	8	0/2	0	10		
Imipramine	10	8	0/8	0	67	>20	n.d.
	20	7	0/5	0	24		
Lisuride	0.039	7	1/7	14	41	0.078	n.d.
	0.078	7	3/6	50	25		
	0.156	8	2/4	(50)	33		

*Number of rats selecting the drug lever divided by the total number of rats completing a selection.

†Values from Table 1.

‡Values in parentheses were not considered for determination of ED_{50} because of low number of subjects responding.

n.d.: not determined.

sant effects. This statement is in general agreement with the tenet that drug discriminative stimuli are best attributed to pharmacological effects of drugs, and are difficult to map onto psychological constructs such as "anxiety" or "depression," let alone therapeutic constructs such as "anxiolytic" or "antidepressant" (1). In this respect, drug discrimination procedures are no different from other behavioural tests in which the difficulty of attributing specific effects of drugs to clinical therapeutic potential has frequently been discussed within the context of behavioural assays of drug action vs. animal models of disease states (23).

Thus, although rolipram is reported to possess antidepressant activity, the interoceptive cue cannot be considered as an antidepressant cue for several reasons. Classical antidepressants, in general, have not proven to be good substrates for the formation of discriminative stimuli [(7), also see (1) for a review]. Furthermore, although rolipram provided a specific interoceptive cue, this did not generalise to other antidepressants, including imipramine [present paper, (13)] and mianserin (24). This might suggest at first glance that rolipram achieves its antidepressant activity through a mechanism different from those of the tricyclic antidepressants, even though it has in common with them the ability to upregulate β-adrenergic receptors following subchronic treatment (18).

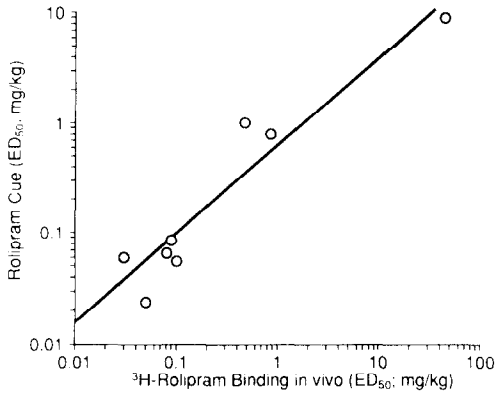

FIG. 3. Comparison of in vivo [^{3}H]rolipram displacement activity from rat forebrain (ED_{50}, mg/kg) and generalisation to the (±)-rolipram cue (ED_{50}, mg/kg) of the drugs listed in Table 2, $N = 8$. Correlation coefficient $r = 0.95$.

One possible explanation of different cueing properties of rolipram and other clinically active antidepressants in the face of similar therapeutic effect might reflect the need for repeated treatments over several weeks for both rolipram and tricyclic antidepressants to achieve their therapeutic effect. Thus, although acute rolipram and tricyclic antidepressants possess different actions that can be expected to give rise to different discriminative stimuli, they may nevertheless in the longer term achieve their therapeutic effects by a common end point, downstream from their initial site of action. The common ability of rolipram and other antidepressants to upregulate β-adrenergic receptors (18) may be a reflection of this common action following long-term treatment. A discriminative stimulus provided by drugs given acutely cannot reflect potential therapeutic effects developing over weeks of treatment. Discriminative stimuli relate to immediate effects, presumably triggered by the same pharmacological mechanism, rather than to similar long-term changes induced by different mechanisms. It may therefore have been more profitable to seek substances giving rise in the short term to rolipram-like effects [e.g., head twitches (20)] as potential substitutes for rolipram in these experiments. Another such possibility is offered by the known interaction of rolipram with central monoaminergic systems (9,18), and it seems likely that the partial substitution of lisuride in the racemate cue might be attributable to a commonality in the actions of PDE inhibitors and direct monoamine agonists in strengthening signals at monoamine-activated G-proteins.

In summary, rolipram and its stereoisomers have been shown to form qualitatively similar discriminative stimuli in rats. Because PDE inhibitors generalise to the discriminative stimulus depending on their ability to interact with the rolipram-sensitive binding site in vivo in rat forebrain, it is likely that the rolipram discriminative stimulus is mediated by an action at this site.

ACKNOWLEDGEMENTS

The authors thank HaTu Lam, Heidi Gartzke, Manuela Grützner and Jörg Seidler for expert technical assistance, and acknowledge the gift of agents from the mentioned drug companies.

REFERENCES

1. Andrews, J. S.; Stephens, D. N. Drug discrimination models in anxiolytic and antidepressant research. In: File, S. E., ed. Psychopharmacology of anxiolytics and antidepressants. New York: Pergamon Press, Inc.; 1991:107–130.
2. Bertolino, A.; Crippa, D.; DiDio, S.; Fichte, K.; Musmeci, G.; Porro, V.; Rapisarda, V.; Sastre-y-Hernandez, M.; Schratzer, M. Rolipram versus imipramine in inpatients with major, "minor" or atypical depressive disorder: A double-blind double-dummy study aimed at testing a novel therapeutic approach. Int. Clin. Psychopharmacol. 3:245–253; 1988.
3. Davis, C. W. Assessment of selective inhibition of rat cerebral cortical calcium-independent and calcium-dependent phosphodiesterases in crude extracts using deoxycyclic AMP and potassium ions. Biochim. Biophys. Acta 797:354–362; 1984.
4. Eckmann, F.; Fichte, K.; Meya, U.; Sastre-y-Hernandez, M. Rolipram in major depression: Results of a double-blind comparative study with amitriptyline. Curr. Ther. Res. 43:291–295; 1988.
5. Fleischhacker, W. W.; Hinterhuber, H.; Bauer, H.; Pflug, B.; Berner, P.; Simhandl, C.; Wolf, R.; Gerlach, W.; Jaklitsch, H.; Sastre-y-Hernandez, M.; Schmeding-Wiegel, H.; Sperner-Unterweger, B.; Voet, B.; Schubert, H. A multicenter double-blind study of three different doses of the new cAMP-phosphodiesterase inhibitor rolipram in patients with major depressive disorder. Pharmacopsychiatry 26:59–64; 1992.
6. Glennon, R. A.; Jacyno, J. M.; Young, R. A comparison of the behavioral properties of (±)-, (−)-, and (+)-5-methoxy-α-methyltryptamine. Biol. Psychiatry 18:493–498; 1983.
7. Jones, C. N.; Howard, J. L.; McBennett, S. T. Stimulus properties of antidepressants in the rat. Psychopharmacology (Berlin) 67:111–118; 1980.
8. Kaulen, P.; Brüning, G.; Schneider, H. H.; Sarter, M.; Baumgarten, H. G. Autoradiographic mapping of a selective cyclic adenosine monophosphate phosphodiesterase in rat brain with the antidepressant [^{3}H]rolipram. Brain Res. 503:229–245; 1989.
9. Kehr, W.; Debus, G.; Neumeister, R. Effects of rolipram, a novel antidepressant, on monoamine metabolism in rat brain. J. Neural Transm. 63:1–12; 1985.
10. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-dependent experiments. J. Pharmacol. Exp. Ther. 96:99–103; 1949.
11. Mulzer, J.; Zuhse, R.; Schmiechen, R. Enantioselective synthesis of the antidepressant rolipram by Michael Addition to a nitroolefin. Angew. Chem. Int. Ed. Engl. 31:870–872; 1992.
12. O'Donnell, J. M. Antidepressant-like effects of rolipram and other inhibitors of cyclic adenosine monophospate phosphodiesterase on behavior maintained by differential reinforcement of low response rate. J. Pharmacol. Exp. Ther. 264:1168–1178; 1993.
13. Ortmann, R.; Meisburger, J. G. Rolipram forms a potent discriminative stimulus in drug discrimination experiments in rats. Psychopharmacology (Berlin) 89:273–277; 1986.
14. Schmiechen, R.; Schneider, H. H.; Wachtel, H. Close correlation between behavioural response and binding *in vivo* for inhibitors of the rolipram-sensitive phosphodiesterase. Psychopharmacology (Berlin) 102:17–20; 1990.
15. Schneider, H. H. Brain cAMP response to phosphodiesterase inhibitors in rats killed by microwave irradiation or decapitation. Biochem. Pharmacol. 33:1690–1693; 1984.
16. Schneider, H. H.; Wachtel, H. Correlation of ^{3}H-rolipram binding to a cerebral cAMP phosphodiesterase with antidepressant activity. In: Jacobson, K. A.; Daly, J. W.; Manganiello, V., eds. Purines in cellular signaling: Targets for new drugs. New York: Springer-Verlag; 1990:303–308.
17. Schneider, H. H.; Schmiechen, R.; Brezinski, M.; Seidler, J. Ste-

reospecific binding of the antidepressant rolipram to brain protein structures. Eur. J. Pharmacol. 127:105–115; 1986.
18. Schultz, J. E.; Schmidt, B. H. Rolipram, a stereospecific inhibitor of calmodulin-independent phosphodiesterase, causes β-adrenoceptor subsensitivity in rat cerebral cortex. Naunyn Schmiedebergs Arch. Pharmacol. 333:23–30; 1986.
19. Spencer, D. G., Jr.; Emmett-Oglesby, M. W. Parallel processing strategies in the application of microcomputers to the behavioral laboratory. Behav. Res. Instrum. Comp. 17:294–300; 1985.
20. Wachtel, H. Characteristic behavioral alterations in rats induced by rolipram and other selective adenosine cylic 3′,5′-monophosphate phosphodiesterase inhibitors. Psychopharmacology (Berlin) 77:309–316; 1982.
21. Wachtel, H. Potential antidepressant activity of rolipram and other selective cyclic adenosine 3′,5′-monophosphate phosphodiesterase inhibitors. Neuropharmacology 22:267–272; 1983.
22. Wachtel, H. Neurotropic effects of the optical isomers of the selective adenosine cyclic 3′,5′-monophosphate phosphodiesterase inhibitor rolipram in rats *in vivo*. J. Pharm. Pharmacol. 35: 440–444; 1983.
23. Willner, P. The validity of animal models of depression. Psychopharmacology (Berlin) 83:1–16; 1984.
24. Yamamoto, T.; Miyamoto, K.; Ueki, S. Rolipram as a discriminative stimuli: Transfer to phosphodiesterase inhibitors. Jpn. J. Pharmacol. 43:165–171; 1987.