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Antagonism of the Effects of the Atypical Benzodiazepine, Ro 5-4864 on Intracranial Self-Stimulation in the Rat

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PELLOW, S , L J HERBERG AND S E FILE *Antagonism of the effects of the atypical benzodiazepine, Ro 5-4864 on intracranial self-stimulation in the rat* PHARMACOL BIOCHEM BEHAV **24**(2) 193–197, 1986 —The effects of Ro 5-4864 (chlordiazepam) were examined on responding for self-stimulation of the mid-lateral hypothalamus Rewarding stimuli were delivered according to a 10-sec variable interval schedule of reinforcement Ro 5-4864 (10–30 mg/kg, subconsulsive doses in these rats) decreased responding This effect was antagonized by chlordiazepoxide (5–10 mg/kg) and phenobarbitone (35 mg/kg) but not by the benzodiazepine receptor antagonist Ro 15-1788 (10–20 mg/kg), the ligand for peripheral benzodiazepine receptors PK 11195 (60 mg/kg) or phenytoin (60 mg/kg) The pattern of interactions of Ro 5-4864 with these compounds differs from the pattern obtained with other procedures, and suggests that Ro 5-4864 has effects on systems unrelated to anxiety, convulsive activity or sedation

Intracranial self-stimulation Ro 5-4864 Benzodiazepine antagonism

Ro 5-4864 is a 1,4-benzodiazepine that, although having negligible affinity for 'classical' CNS benzodiazepine receptors, binds with high affinity to 'peripheral-type' benzodiazepine binding sites that are present in the periphery [4] and in the CNS [27] Although Ro 5-4864 has no benzodiazepine-like activity in animal tests [31] and thus has long been considered inactive [24], recent investigations into its behavioural effects have shown that it has convulsant [9, 22, 25, 30], anxiogenic [8, 11, 12, 19] and sedative [10] properties Further behavioural, electrophysiological and biochemical investigations have questioned the conclusion, made on the basis of binding studies, that Ro 5-4864 does not interact with the GABA/benzodiazepine/ionophore complex in the CNS, and suggest a possible action at the picrotoxinin site that is linked with the chloride ionophore on this complex (see [19,20] for reviews)

Responding for lateral hypothalamic self-stimulation on a variable interval schedule of reinforcement has been shown to be a sensitive procedure for identifying and distinguishing the behavioural effects of benzodiazepines, GABA agonists and antagonists, non-GABAergic anticonvulsants [13] and benzodiazepine 'antagonists' such as Ro 15-1788, CGS 8216 and FG 7142 [23,24] Our aim was to characterize further the behavioural effects of Ro 5-4864 using this procedure, and to examine its profile in combination with several other compounds with which it has previously been shown to interact These were PK 11195, an isoquinoline carboxamide derivative that, like Ro 5-4864, selectively binds to peripheral-type benzodiazepine binding sites [15,16] and is thought to be an antagonist there [17], chlordiazepoxide, a conventional 1,4 benzodiazepine with moderate potency for classical CNS benzodiazepine receptors but with negligible affinity for peripheral-type sites [27], Ro 15-1788, an imidazodiazepine that antagonizes several of the effects of benzodiazepines and β-carbolines [14,21] and that is believed to interact only with classical CNS benzodiazepine receptors [26], phenobarbitone, a barbiturate that displaces [^{3}H]-α-dihydropicrotoxinin and [^{35}S]-t-butyl bicyclophosphorothionate from the picrotoxin site, and phenytoin, which interacts with benzodiazepines at low affinity binding sites in the brain [2, 3, 7, 18, 28] Doses of chlordiazepoxide, Ro 15-1788, phenobarbitone and phenytoin were chosen on the basis of previous studies ([13,23], Pellow and File, unpublished), dose-response data were determined for Ro 5-4864 and PK 11195

METHOD

Animals

Male hooded PVG rats (Bantin and Kingman Ltd) weighing 230–250 g at time of surgery were individually housed with free access to food and water

Under halothane anesthesia, twisted bipolar stainless steel electrodes (0 25 mm diameter, Plastic Products Inc ,

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TABLE 1

MEAN (+S E M) SELF-STIMULATION RATE (% BASELINE) IN EACH 15-MIN PERIOD OF A 60-MIN TEST SESSION, IN RATS TESTED IMMEDIATELY AFTER IP INJECTION WITH Ro 5-4864 (10 AND 30 mg/kg) OR PK 11195 (10–60 mg/kg)

Drug	1st 15-min	2nd 15-min	3rd 15-min	4th 15-min
control	98 2 ± 1 03	99 8 ± 1 67	97 5 ± 3 61	101 3 ± 4 17
Ro 10	95 2 ± 2 51	92 6 ± 3 77	95 2 ± 4 55	99 5 ± 5 03
Ro 30	72 8 ± 6 16*	68 1 ± 6 56*	58 9 ± 10 4*	63 4 ± 10 8*
PK 10	95 2 ± 4 43	99 7 ± 4 59	101 5 ± 6 97	96 2 ± 10 10
PK 30	92 8 ± 3 75	91 8 ± 13 4	98 4 ± 3 43	100 6 ± 8 08
PK 60	87 4 ± 7 23	89 4 ± 7 50	86 9 ± 8 00	95 5 ± 12 20

*$p<0$ 05

Roanoke, VA) were stereotaxically implanted in the midlateral hypothalamus 1 0 mm posterior to Bregma, 1 3 mm to the left of the midline and 8 0 mm below the skull surface Electrode placements were verified on enlarged photographic projects of unstained 50-μm frozen sections at the end of the investigation

Drugs

Ro 5-4864 (Hoffman-La Roche), PK 11195 (Pharmuka), phenytoin (Sigma), sodium phenobarbitone (BDH Chemicals) and Ro 15-1788 (Hoffman-La Roche) were suspended in distilled water with a drop of Tween 20 and dispersed by ultrasound, chlordiazepoxide hydrochloride (Roche Products) was dissolved in distilled water Control injections consisted of distilled water, with or without a drop of Tween 20, as appropriate Drugs were injected intraperitoneally in concentrations to give an injection volume of 2 ml/kg

Self-Stimulation

Rats were allowed to recover from surgery for a week, and then trained to operate a pedal for a 0 5-sec 50-Hz sinewave reinforcing impulse available on a variable interval schedule of 10-sec mean duration (see [13, 23, 24] The stimulating current for each rat was fixed at the lowest intensity that elicited uninterrupted responding in preliminary trials in which intensities were decremented in decilog steps, between 50 and 10 μA r m s Response rates in different rats ranged between 15 and 25 lever-presses/min

During test sessions, animals were allowed to self-stimulate for 45 min, of which the last 30 min provided a pre-injection baseline The rat was then injected, and allowed to respond for a further 75 min If the rat stopped responding it was encouraged to restart by taps on the lever, if this failed, by administration of priming shocks, and, finally, by the experimenter placing the rat bodily on the lever Response rates were recorded automatically at 5-min intervals as a digital printout Drug effects were determined from the rate recorded during each 15-min period following injection, expressed as a percentage of pre-injection baseline, and compared with the corresponding rate after control injections

Procedure

Experiment 1 Dose response data were obtained for Ro 5-4864 and PK 11195 on self-stimulation responding Drug groups were vehicle, Ro 5-4864 10 and 30 mg/kg, PK 11195 10, 30 and 60 mg/kg (n=7 per group)

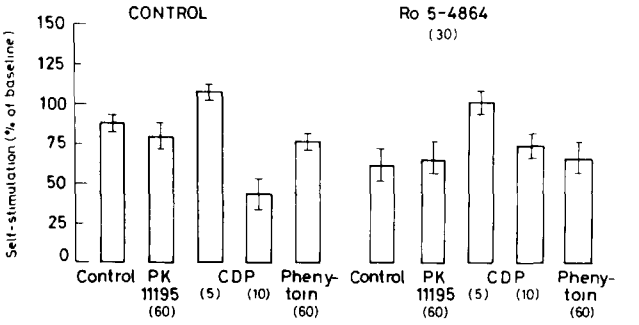

FIG 1 Mean (±S E M) self-stimulation rate (variable interval 10 sec) in the second 15-min period after injection for rats given phenytoin (60 mg/kg) or chlordiazepoxide (5 or 10 mg/kg) alone or in combination with Ro 5-4864 (30 mg/kg)

Experiment 2 The effects of an active dose of Ro 5-4864 (30 mg/kg) were investigated in combination with phenytoin (60 mg/kg), phenobarbitone (35 mg/kg), Ro 15-1788 (10 and 20 mg/kg), chlordiazepoxide (5 and 10 mg/kg), PK 11195 (60 mg/kg) (n=6 per group)

Each rat was tested at 48-hr intervals, and in each experiment the drug treatments were administered in a predetermined random order that was different for each rat

Statistics

Data from Experiment 1 were analysed by Friedman 2-way analysis of variance, and comparisons between individual groups were made with Wilcoxon matched-pairs signed-ranks tests Data from Experiment 2 were analysed by Wilcoxon matched-pairs signed-ranks tests

RESULTS

Experiment 1

Ro 5-4864 (10–30 mg/kg) caused a significant dose-related reduction in responding for self-stimulation in the first, $\chi^2=6$ 0, $p<0$ 05, second, $\chi^2=12$ 29, $p<0$ 005, third, $\chi^2=8$ 86, $p<0$ 05, and fourth, $\chi^2=10$ 57, $p<0$ 005, 15-min time periods after injection (see Table 1) Wilcoxon tests showed that at 30 mg/kg significant reductions in self-stimulation respond-

TABLE 2

SUMMARY TABLE OF THE ABILITY OF SEVERAL COMPOUNDS TO ANTAGONISE THE EFFECTS OF Ro 5-4864 IN ANIMAL TESTS OF ANXIETY, CONVULSIVE ACTIVITY, EXPLORATION AND SEDATION, AND IN THE PRESENT SELF-STIMULATION PROCEDURE

Drug	Anxiety	Convulsions	Exploration	Motor	ICSS
Benzodiazepines	↓ [11]	↓ [7,29]	?	?	↓
Barbiturates	↓ *	↓ [29]	?	?	↓
Ro 15-1788	– [11]	↓ [7]	– [10]	– [10]	–
PK 11195	– [8,12]	↓ [6,8]	↓ [10]	– [10]	–
Phenytoin	↓ [8]	↓ [8]	– [10]	– [10]	–

*Pellow and File, unpublished data

↓ compound antagonises Ro 5-4864, – compound does not affect Ro 5-4864, ? not yet reported References to studies are in brackets (see text)

ing were observed at all time-points PK 11195 had no significant effect at any time period, χ^2=2 49, 2 48, 0 09, 0 43, respectively), see Table 1

Experiment 2

Phenytoin (60 mg/kg) significantly decreased self-stimulation in the second 15-min period after injection ($p<0$ 05) but did not antagonize the reduction produced by Ro 5-4864 (30 mg/kg) (see Fig 1) CDP increased self-stimulation at 5 mg/kg ($p<0$ 05) and decreased it at 10 mg/kg ($p<0$ 05), and at both doses significantly reversed the reduction produced by Ro 5-4864 (30 mg/kg, $p<0$ 05, see Fig 1) Interestingly, however, Ro 5-4864 did not antagonize the increase in self-stimulation produced by CDP (5 mg/kg) PK 11195 (60 mg/kg) had no significant effect on self-stimulation alone, and did not antagonize the effects of Ro 5-4864 (see Fig 2) Ro 15-1788 (10–20 mg/kg) did not antagonize the effects of Ro 5-4864, although at 20 mg/kg this compound when given alone significantly increased self-stimulation (see Fig 2) Phenobarbitone (35 mg/kg) significantly increased self-stimulation ($p<0$ 05), and also antagonized the reduced self-stimulation produced by Ro 5-4864 (30 mg/kg, $p<0$ 05, see Fig 2)

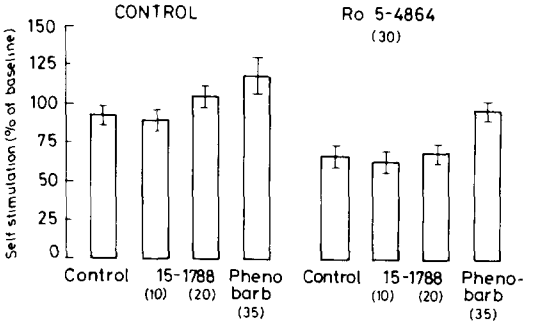

FIG 2 Mean (±S E M) self-stimulation rate (variable interval, 10 sec) in the second 15-min period after injection for rats given PK 11195 (60 mg/kg) and Ro 15-1788 (10 or 20 mg/kg) alone or in combination with Ro 5-4864 (30 mg/kg)

DISCUSSION

Ro 5-4864 at 30 mg/kg produced a decrease in responding for lateral hypothalamic self-stimulation, similar to that reported for the benzodiazepine receptor 'inverse agonists' CGS 8216 and FG 7142 [23,24] Since Ro 5-4864 is a compound with known convulsant actions, it is necessary to consider the extent to which these actions could account for its effects on self-stimulation The dose of Ro 5-4864 that has been found to effectively cause convulsions has varied considerably, both between (c f [1, 6, 25, 29]) and within (cf [6, 7, 22]) laboratories In our experience, differences have been observed between species (cf. [6,7]) and strains (cf [6,9]), and also between different batches of the supplied compound In each new batch of experiments, then, we have found it necessary to establish the effective convulsant dose of Ro 5-4864 before carrying out the intended behavioural work During the course of the present study we observed, in this strain of rats, that convulsions did not occur at doses below 90 mg/kg At 90 mg/kg 100% rats convulsed, at 60 mg/kg we only observed myoclonic spasms in one rat who had been treated and tested for self-stimulation responding In no cases were convulsant-like activities observed at 30 mg/kg It is therefore unlikely that the convulsant properties of Ro 5-4864 were contributing to its effects on self-stimulation The pattern of interactions of Ro 5-4864 with other drugs (see below and Table 2) supports this view

PK 11195 alone (10–60 mg/kg) had no significant effect on self-stimulation, higher doses were not investigated since at the 60 mg/kg dose some animals showed unusual body postures that could have interfered with their ability to lever-press The reduction in self-stimulation produced by Ro 5-4864 (30 mg/kg) was not antagonized by PK 11195 (60 mg/kg), this is consistent with the inability of PK 11195 to antagonize the effects of Ro 5-4864 in the social interaction and punished drinking procedures [12], although PK 11195 does antagonize the proconvulsant and convulsant effects [1,9] and the reductions in exploratory head-dipping produced by Ro 5-4864 [10]

As found previously [13, 23, 24], chlordiazepoxide (5 but not 10 mg/kg) increased self-stimulation Chlordiazepoxide at both doses significantly reversed the reduction in response rates produced by Ro 5-4864, however, interestingly, Ro 5-4864 was unable to antagonize the increase in self-stimulation produced by chlordiazepoxide (5 mg/kg) Chlordiazepoxide is able to antagonize the anxiogenic effects of Ro 5-4864 [11], and other benzodiazepines have been found to antagonize the convulsant effects of this compound [7,29] This is similar to the effects obtained by Herberg and Williams [13] with picrotoxin and chlordiazepoxide, however, unlike picrotoxin, Ro 5-4864 did not produce a further

enhancement of responding when combined with chlordiazepoxide (10 mg/kg)

Ro 15-1788 (20 mg/kg) enhanced self-stimulation, as previously found by Pellow *et al* [23] This compound did not antagonize the reduction in self-stimulation produced by Ro 5-4864, again consistent with findings in the social interaction test of anxiety and the holeboard [10,11] However, Ro 15-1788 (20 mg/kg) is able to antagonize the convulsions produced by Ro 5-4864 in at least some circumstances [7] Phenobarbitone (35 mg/kg), like the barbiturate phenobarbitone [13], increased self-stimulation and antagonized the effects of Ro 5-4864 Again, similar results have been obtained in the social interaction test (Pellow and File, unpublished), where a dose of phenobarbitone that also increased social interaction was able to reverse the decrease produced by Ro 5-4864 However, it is not possible to be sure whether the antagonism between these two compounds in both of these tests is specific or whether it simply reflects a cancellation of two opposite intrinsic actions Barbiturates also prevent the convulsions usually observed with Ro 5-4864 [29]

Consistent with the report of Herberg and Williams [13], phenytoin decreased self-stimulation The inability of phenytoin (60 mg/kg) to antagonize the decrease in self-stimulation produced by Ro 5-4864 contrasts with its ability to antagonize the anxiogenic and convulsant actions of this compound [8,9] but is similar to its inability to antagonize the reductions in exploratory head-dipping and locomotor activity in the holeboard produced by Ro 5-4864 [10] It is unlikely that the depressant effect that phenytoin produces when given alone would interfere with its ability to antagonize the reduction produced by Ro 5-4864, since chlordiazepoxide at a dose that decreased response rates (10 mg/kg) was still able to antagonize the reduction produced by Ro 5-4864

In conclusion, the pattern of interaction of Ro 5-4864 with other compounds in the variable-interval self-stimulation procedure shows a broad similarity to the patterns seen in various other procedures, but there are also some notable differences (see Table 2) It is thus uncertain whether the effects of Ro 5-4864 in this procedure are to be attributed to its effects on anxiety, convulsant activity or sedation, or to its effects on other, unrelated systems The same question applies to several related agents that have also been found to depress self-stimulation, such as the benzodiazepine receptor 'inverse agonists' FG 7142 and CGS 8216, and the receptor antagonist Ro 15-1788 [23,24] Experiments are currently underway to investigate this question

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