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The Effects of β-Carboline Carboxylic Acid Ethyl Ester and its Free Acid, Administered ICV, on the Anticonvulsant Activity of Diazepam and Sodium Valproate in the Mouse

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VELLUCCI, S V AND R A WEBSTER *The effects of β-carboline carboxylic acid ethyl ester and its free acid, administered ICV, on the anticonvulsant activity of diazepam and sodium valproate in the mouse* PHARMACOL BIOCHEM BEHAV **24**(4) 823–827, 1986 —The effects of intracerebroventricular (ICV) β-carboline carboxylic acid ethyl ester (β-CCE) and its free acid on the protective effects of diazepam against leptazol- and R05-3663-induced convulsions were investigated in mice and compared with their effects on the antileptazol effect of sodium valproate, in an attempt to demonstrate a specific central effect of β-CCE on benzodiazepine function The results show that a small dose (1 μg) of β-CCE but not its free acid (in doses of up to 100 μg) was able to reverse the protective effects of diazepam against leptazol- and R05-3663-induced convulsions, whereas the effects of sodium valproate, a non-benzodiazepine anticonvulsant, could not be reversed by these β-carboline derivatives

β-Carboline carboxylic acid ethyl ester (β-CCE) Diazepam Sodium valproate Convulsions Mice

FOLLOWING the isolation of β-carboline carboxylic acid ethyl ester (β-CCE) from human urine and mammalian brain, and the discovery that this compound is a potent and specific inhibitor of benzodiazepine (Bz) binding to rat brain membrane fragments *in vitro* (K_i~1 nm [2, 12, 13]), it was established that β-CCE administered via a peripheral route could counteract the pharmacological effects of Bzs *in vivo*. For example, β-CCE was shown to antagonize the anticonvulsant effects of diazepam [15,23], lower the seizure threshold to bicuculline and antagonize the locomotor inhibitory effects of flurazepam [4], as well as counteracting the anxiolytic activity of Bz [3]. These studies involved the administration of β-CCE intravenously in high doses (relative to its potency), as the compound is rapidly hydrolyzed by liver and kidney esterases [19,20]. More recently we have carried out experiments in which low (μm) doses of β-CCE were administered intracerebroventricularly (ICV) in order to avoid possible metabolic and indirect effects and demonstrated that these low doses were capable of antagonizing the anticonflict effects of chlordiazepoxide in the rat [26] and of antagonizing the antileptazol effects of diazepam and exerting a proconvulsant effect in the mouse [25].

In the present work some of the latter observations have been extended in order to attempt to demonstrate a specific central effect of β-CCE on Bz receptor mediated effects. Thus the ability of β-CCE and its free acid, which has significantly lower affinity for brain Bz receptors than β-CCE [2], administered ICV to conscious mice, to counteract the protective effects of diazepam against leptazol- and R05-3663-induced convulsions was investigated and compared with their effects on the antileptazol activity of sodium valproate, a non-benzodiazepine anticonvulsant drug.

METHOD

Female C3H mice (20–30 g body weight) were used in randomized groups allocated to new cages not less than 2 hr before the start of the experiments. Each animal was used for one experiment only. For the ICV injections a standard procedure was employed in which 10 μl vol. were slowly injected into the third cerebral ventricle of the intact conscious mouse via a Hamilton syringe with a 27 gauge needle 3.5 mm in length. At the end of each experiment the animals were killed, the skull exposed and the injection site verified.

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TABLE 1

THE EFFECTS OF β-CCE AND ITS FREE ACID ON THE ANTI-LEPTAZOL AND ANTI-RO5-3663 ACTIVITY OF DIAZEPAM

	Treatment									
	Vehicle IP + ACSF or β-CCE Vehicle ICV		Diazepam (500 μg/kg) IP + β-CCE Vehicle ICV		Diazepam (500 μg/kg) IP + β-CCE (0 5 μg) ICV		Diazepam (500 μg/kg) IP + β-CCE (1 μg) ICV		Diazepam (500 μg) IP + β-Carboline Carboxylic Acid 1 μg, 10 μg, 100 μg ICV	
	% convulsing (n)		% convulsing (n)		% convulsing (n)		% convulsing (n)		% convulsing (n)	
Leptazol (60 mg/kg) IP	100%	(30)	0*	(20)	75%†	(12)	100%†	(10)	0	(18)
RO5-3663 (15 mg/kg) IP	100%	(12)	0*	(12)	30%	(10)	100%†	(8)	0	(18)

Diazepam was administered 15 min before, and the β-carboline derivatives were administered immediately before, the convulsant drugs

*$p<0.001$ compared with the response to convulsant alone

†$p<0.001$ compared with the response in the absence of β-CCE, (Fisher exact probability test)

There was no significant difference in the time to fullbody convulsion in animals treated with ACSF and leptazol (60 mg/kg) and those treated with β-CCE (1 μg) and leptazol (60 mg/kg)

TABLE 2

THE EFFECT OF β-CCE AND ITS FREE ACID ON THE ANTI-LEPTOZOL ACTIVITY OF SODIUM VALPROATE

	Treatment									
	Vehicle IP + ACSF or β-CCE Vehicle ICV		Sodium Valproate (200 mg/kg) IP + β-CCE Vehicle ICV		Sodium Valproate (200 mg/kg) + β-CCE 0 5 μg		Sodium Valproate (200 mg/kg) + β-CCE 1 μg		Sodium Valproate (200 mg/kg) + β-Carboline Carboxylic Acid (100 μg)	
	% Convulsing (n)		% Convulsing (n)		% Convulsing (n)		% Convulsing (n)		% Convulsing (n)	
Leptazol (60 mg/kg) IP	100%	(30)	0*	(6)	0*	(6)	0*	(6)	0*	(6)

Sodium valproate was administered 15 min before, and the β-carboline derivatives were administered immediately before the convulsant drugs

*$p<0.001$ compared with the response to convulsant alone (Fisher exact probability test)

Results from animals with incorrectly placed injections were discarded

In order to assess convulsant activity, the mice were placed singly, in perspex boxes (23×27×16 5 cm) and the time taken for the onset of generalised myoclonic seizures following the administration of the convulsant drug was recorded The animals were observed for 20 min and scored either as "convulsing" or "not convulsing " Those that convulsed were killed immediately by cervical dislocation

Statistical analysis of the data was performed using the Fisher exact probability test The following drugs were used· Diazepam (Valium, Roche Products Ltd.) was dissolved in a minimum volume of propylene glycol in distilled water and diluted with 0.9% saline This was administered in a dose of

500 μg/kg, IP. Sodium valproate (Epilim, Labaz) was dissolved in saline and administered IP in doses of up to 300 mg/kg. Leptazol (pentylenetetrazol, Sigma Chemical Co. Ltd.) was dissolved in 0.9% saline and administered IP. R05-3663 (1,3,dihydro-5-methyl-2H1, 4-benzodiazepine-2-one, a convulsant benzodiazepine derivative, Roche Products Ltd.) was dissolved in 0.3 ml of warm ethanol, made up to 10 ml with distilled water and also administered IP. To prepare the β-carboline derivatives (i.e., β-carboline-3-carboxylic acid ethyl ester and the corresponding free acid), approximately 0.1 ml acetic acid was added to a weighed amount of the compound followed by 5 ml distilled water. The pH of the solution was carefully adjusted to 6.5 with 2 N NaOH and the final volume made up to 10 ml with distilled water so that 10 μl of the final solution contained the dose to be administered ICV.

Control treatments consisted of equal-volume injections of appropriate vehicles (i.e., 1 ml/100 g for IP and 10 μl for ICV administration)

RESULTS

The doses of leptazol and R05-3663 that were used here (i.e., 60 and 15 mg/kg, respectively) were the lowest doses of the compounds that produced full-body convulsions (i.e., generalized myoclonic seizures) in 100% of the animals within 3–4 min. These effects could be completely antagonized by diazepam (500 μg/kg) administered 15 min prior to the convulsant drug. The free carboxylic acid of β-CCE (in doses of 1–100 μg ICV) and the vehicle had no behavioural effects, whereas β-CCE (0.5 and 1 μg ICV) produced behavioural changes in some animals which included increased locomotor activity but there was no evidence of an overt convulsant effect.

β-CCE (0.5 and 1 μg) administered 15 minutes after diazepam and immediately before leptazol significantly ($p<0.001$) reduced the antileptazol effect of diazepam in a dose-related manner (Table 1). On the other hand, the free acid (in doses of up to 100 μg) failed to reverse diazepam's antileptazol effect and no convulsions were observed. Similarly, β-CCE (1 μg) administered 15 minutes after diazepam, and immediately before R05-3663, significantly ($p<0.001$) antagonized diazepam's anticonvulsant effect whereas the free acid was ineffective (Table 1).

The antileptazol effects of sodium valproate were not affected by β-CCE (1 μg) despite the fact that the dose of valproate chosen was one which only just blocked the effects of leptazol Thus body twitches were noted in animals treated with valproate and leptazol (both in the presence and absence of β-CCE), however none of the animals convulsed and the incidence of these twitches was similar in both groups (Table 2).

Although β-CCE has been known to potentiate the effects of low (subconvulsant) doses of leptazol, there is no evidence to indicate that this was the case with the (convulsant) dose of leptazol used in the present work. The latencies to full-body convulsions following leptazol (60 mg/kg, IP) in the absence and presence of β-CCE (1 μg, ICV) were 3.42±0 28 and 2.9±0.78 minutes, respectively, with all the animals (n=20) convulsing in each group.

DISCUSSION

These results show that a low (1 μg) dose of β-CCE, administered directly into the third ventricle of the mouse brain, is capable of antagonizing the antileptazol and anti-R05-3663 effects of diazepam, whereas the corresponding carboxylic acid, in doses of up to 100 μg ICV or its vehicle, were inactive in this respect. Although the injection sites were not examined histologically but were verified by inspection, it is considered unlikely that the convulsions occurring in β-CCE-treated animals were due to tissue damage caused by the injection or injected substance, as no evidence of convulsant activity was noted in the corresponding vehicle-treated controls or after treatment with the free acid.

Following the ICV administration of a drug the extent to which cerebral diffusion occurs may be crucial in determining any effects which it may produce. It is possible, though unlikely, that the observed lack of effect of the free acid compared with that of β-CCE may have been due to differences in diffusion patterns and hence CNS sites reached by the two substances after ICV administration. This question may only be resolved satisfactorily by means of autoradiographic control studies. The observation that β-CCE, but not the free acid, could reverse the anticonvulsant effects of diazepam correlates well with the observations *in vitro* which indicate that the IC_{50} of β-CCE for the inhibition of specific [^{3}H] flunitrazepam binding is 7 nM, whereas that of the free acid is 31 μM [2].

β-CCE is highly lipophilic and therefore expected to readily cross the blood-brain barrier, however, it has been demonstrated that the ability of this compound and related esters to occupy Bz receptors in the mouse brain *in vivo* is substantially less than that observed *in vitro* [19]. Peripherally administered β-carboline carboxylic acid esters are rapidly hydrolysed by liver and kidney esterases, whereas the brain is almost completely devoid of such activity Thus the ICV route is a very useful one for the administration of these compounds as it eliminates the possibility that β-CCE may be having indirect effects and renders unneccessary the use of the high doses (relative to its potency) which have to be employed when the compound is administered peripherally

Our findings are in agreement with the observations of Tenen and Hirsch [23] who administered β-CCE intravenously to mice (in doses of 3.2–32 mg/kg) and demonstrated that it could antagonize the antileptazol effect of diazepam. A similar finding was reported by Oakley and Jones [15] who administered β-CCE to mice in a dose of 100 mg/kg, IP, β-CCE is also capable of potentiating the effects of low (subthreshold) doses of leptazol in mice [15,25], of lowering the seizure threshold to bicuculline [4], of eliciting EEG seizures in the rat [20] and of facilitating the occurence of audiogenic seizures in susceptible mice when exposed to subthreshold sound levels [6]. Thus it is quite possible that the reversal of diazepam's antileptazol effect might have resulted from the proconvulsant activity of β-CCE, rather than from a direct competitive antagonism of the effect of diazepam. However, the former is unlikely as the present work indicates that β-CCE (1 μg) was unable to reverse the effects of a dose of sodium valproate which only just blocked the effects of the dose of leptazol used. Furthermore it has been demonstrated that a similar dose of β-CCE did not potentiate the effects of convulsant doses (60 mg/kg and above) of leptazol in mice (Vellucci, unpublished observations)

Sodium valproate is an anticonvulsant drug which is believed to enhance GABA-mediated neurotransmission by acting at the dihydropicrotoxinin (picrotoxin) site to prolong the life-time of GABA-receptor regulated chloride ionophores [20] There is no evidence to indicate that sodium

valproate acts directly on GABA receptors in a manner similar to bicuculline, as it fails to affect [^{3}H]-muscimol binding [8]. Some authors have demonstrated that R015-1788 could abolish the immobility and sedation produced by valproate, could antagonize some of its electrophysiological effects and could exert a short-lasting antagonistic effect on its anticonflict activity [10,11] However, other authors have been unable to confirm these observations [14,29] and it appears unlikely that the anticonvulsant and anticonflict effects of valproate are mediated via a direct action on Bz receptors, as the drug does not alter basal or GABA receptor-stimulated [^{3}H] diazepam binding to rat brain membrane fragments [24] The present results, which demonstrated that β-CCE was unable to reverse the antileptazol effects of a low dose of valproate, are in agreement with this.

The doses of leptazol and R05-3663 used were chosen on the basis of extensive pilot studies as being equi-effective at producing convulsions in all animals within 5 minutes and which could be completely blocked by 500 μg/kg diazepam Although extensive dose-response studies were not carried out, it appears that the anti-R05-3663 effects of diazepam were less suspectible to β-CCE (0 5 μg) than were its antileptasol effects This difference may be associated with the different proposed sites of action of the two convulsants Leptasol is believed to exert effects at GABA and Bz receptors [9,17] although there is some evidence that it may act at the chloride ionophore site [18], whereas R05-3663, although structurally very similar to diazepam , does not act on Bz receptors but acts specifically at the chloride ionophore (dihydropicrotoxinin) site [5, 7, 16] and shows very little interaction with binding sites for GABA, Bz or β-carbolines [1, 21, 22]

In conclusion, these results indicate that small doses of β-CCE but not its free acid, administered centrally, are capable of specifically reversing the protective effects of diazepam against leptazol- and R05-3663-induced convulsions, whereas the antileptazol activity of sodium valproate, which does not act on Bz receptors, was unaffected

Although the data presented here have been obtained with female mice, we have found that when assessing gross behavioural effects such as the occurrence of generalized myoclonic seizures, that data obtained with male mice are quantitatively and qualitatively similar [25, 27, 28]

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