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CHOLECYSTOKININ OCTAPEPTIDE (CCK-8), CERULETIDE AND ANALOGUES OF CERULETIDE: EFFECTS ON TREMORS INDUCED BY OXOTREMORINE, HARMINE AND IBOGAINE A COMPARISON WITH PROLYL-LEUCYLGLYCINE AMIDE (MIF), ANTI-PARKINSONIAN DRUGS AND CLONAZEPAM

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Summary—Cholecystokinin octapeptide (CCK-8), ceruletide (caerulein, CER) and 10 analogues of ceruletide, were studied in mice for antagonism of the tremors induced by harmine (5 mg/kg, s.c.), ibogaine (20 mg kg, s.c.) and oxotremorine (0.2 mg/kg, s.c.). The following reference drugs were tested for comparison: prolyl-leucylglycine amide (MIF), atropine, haloperidol, biperiden, ethopropazine, trihexyphenidyl, methixene and clonazepam. All treatments were subcutaneous, the antagonists being given 10 min (in some trials 30 min) before the tremorogen. Tremorolytic potency (ED_{50}) was calculated from dose–response curves. Against the tremors induced by either harmine or ibogaine, CCK-8 and ceruletide, as well as many of the analogues of ceruletide had greater tremorolytic potency than the reference drugs. Against oxotremorine, however, ceruletide and its most potent analogue, Nle^8-CER (other analogues were not tested) were inactive and MIF showed very little effectiveness. Additional experiments on hypothermia and sedation as well as evaluation of previous studies on other central actions suggested that the tremorolytic effect of CCK-like peptides is independent of other central effects. The CCK-like peptides may play a physiological role in the regulation of extrapyramidal motor activity.

Key words: tremor, harmine, ibogaine, oxotremorine, cholecystokinin octapeptide (CCK-8), ceruletide, anti-parkinson drugs, clonazepam.

Cholecystokinin octapeptide (CCK-8), ceruletide (caerulein, CER) and analogues of ceruletide produce many central effects, i.e. antinociception, behavioural sedation and an elevation of the threshold for chemically-induced convulsions (Jurna and Zetler, 1981; Zetler, 1980a, b, c, 1981c, d). An unusual feature is the combination of pharmacological properties which are in part benzodiazepine-like (antagonism of picrotoxin, thiosemicarbazide and harman), and in part neuroleptic-like (production of catalepsy and ptosis, antagonism of methylphenidate-induced stereotypy). Nevertheless, pharmacological methods have dissociated the effects of the peptide from those of diazepam and haloperidol (Zetler, 1981a, b, 1982a). Although observing the motor behaviour of the animals yielded knowledge of the pharmacological profile, it is not known if the peptides would modify experimental tremors. This question is important for two reasons. First, neuropeptides and especially cholecystokinin are assumed to play a physiological role in the regulation of extrapyramidal motor-function (for reviews see Joliceur, Rondeau, St-Pierre, Rioux and Barbeau, 1981; Morley, 1982) and one peptide (L-prolyl-L-leucylglycine amide, MIF) has been (not unequivocally) found to possess some tremorolytic activity and to improve the symptoms of Parkinsonism in man (for literature see Gerstenbrand, Poewe, Aichner and Kozma, 1979; Dickinson and Slater, 1982). Second, crude preparations of substance P (from brain), later shown to contain large amounts of CCK-8-like immunoreactive "impurities" (Zetler, Cannon, Powell, Skrabanek and Vanderhaeghen, 1979), antagonized the harmine-induced tremor (Zetler, 1956) possibly because of their content of CCK-8 rather than of substance P.

Therefore, it was necessary to find out whether ceruletide and related peptides antagonized in mice the experimental tremors produced by oxotremorine and the indole alkaloids, harmine and ibogaine. The last compound is (like harmine) a hallucinogen of plant origin (Schultes, 1969) and was used since its neuropharmacological activity (except tremor) differs from that of harmine (Zetler, 1964). Pharmacokinetics and tremorogenic effects of ibogaine (in the mouse) are well-known (Zetler, Singbartl and Schlosser, 1972; Singbartl, Zetler and Schlosser, 1973), so it was hoped that the ibogaine-induced tremor would provide another tool to characterize tremorolytic effects. The actions of the peptides were compared with those of atropine, haloperidol and several anti-Parkinsonian drugs. Clonazepam was also tested because of (a) its powerful clinical and

experimental effect against convulsions and involuntary movements, such as intention myoclonus (Browne, 1976; Goldberg and Dorman, 1976; Chung Hwang and Van Woert, 1979; Menon, Vivonia and Haddox, 1981), (b) the antagonism by diazepam of harmaline-induced tremor (Mao, Guidotti and Costa, 1975) and (c) its solubility in saline (this allowed the diazepam solvent which had turned out to be tremorolytic to be avoided). Finally, MIF was used as the sole peptide for which experience exists in man with Parkinsonism (Gerstenbrand *et al.*, 1979).

METHODS

Animals

Male mice (weighing about 25 g) of the NMRI strain were used throughout (each animal used only once). The mice were kept at an ambient temperature of 23°C and had free access to pellet food and tap water.

Tremors

After the subcutaneous injection of a tremorogen, each mouse was put in an individual glass cylinder (16 cm in height, 11 cm in diameter) on filter paper. The presence or absence of tremors was recorded every 5 min by observing the animal for 30 sec. The period of tremor testing lasted 30 min. For each tremorogen the dose used was large enough to produce tremors in 100% of the mice 5, 10 and 15 min later (Fig. 1). The tremorogenic doses (mg/kg, s.c.) were for harmine, 5; for ibogaine, 20; for oxotremorine, 0.2. According to the experiments shown in Fig. 1, it appeared appropriate to evaluate the first 3 observation times, i.e. the first 15 min (note that saline was administered subcutaneously 10 min before a tremorogen). In order to obtain the all-or-none responses needed for the determination of the ED_{50}, an animal was considered showing a tremorolytic effect if it had no tremor at all, or only during 1 of the 3 steps of observation. Drugs to be tested for tremorolytic activity were mostly given (s.c.) 10 min before a tremorogen. When the waiting time was 30 min (Table 3) the tremorogens had the same efficacy in saline-treated mice, so that it was not necessary to alter the evaluation.

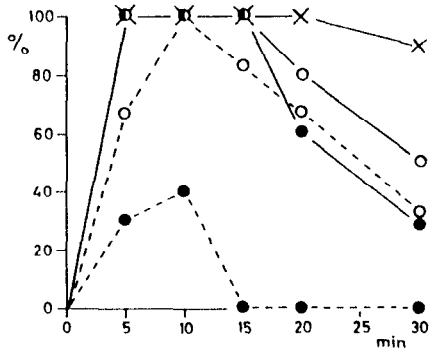

Fig. 1. Time-course of the tremorogenic effect of harmine, ibogaine and oxotremorine in mice after pretreatment with saline (injected subcutaneously 10 min earlier). Abscissae: time (min) following subcutaneous administration of a tremorogen. Ordinate: percentage of mice showing tremor. Harmine: (●——●) 5 mg/kg ($n = 10$); (●-----●) 2.5 mg/kg ($n = 10$). Ibogaine: (○——○) 20 mg/kg ($n = 10$); (○-----○) 10 mg/kg ($n = 6$). Oxotremorine: (×) (0.2 mg/kg ($n = 10$).

Body temperature

Before giving an animal the first injection and after the end of the observation (30 min), the body temperature was measured by an electric thermometer and a probe inserted approximately 18 mm from the anus for at least 5 sec. The temperature in the laboratory was between 22.5 and 23.5 C.

Ptosis and inhibition of exploratory rearing

Ten minutes after administration (s.c.) of either saline or a drug to be tested, each animal was seated in the glass cylinder (see above) and observed for 15 min. Palpebral ptosis was scored by the method of Rubin, Malone, Waugh and Burke (1957) after the first 5 min. For the calculation of the ED_{50}, a mouse with the score 2 (eyes half-closed) was considered ptotic. The number of rearings performed by each mouse within 15 min was counted. The mean rearing activity of 10 saline-treated mice amounted to 54 ± 21 ($\bar{x} \pm SD$) rearings. A mouse was considered to show a reduction in rearing activity if the number of rearings was less than 12, i.e. less than $\bar{x} - 2SD$.

Statistics

The ED_{50}, i.e. the dose that would produce a definite effect in 50% of the mice, was determined according to Litchfield and Wilcoxon (1949). Analysis of variance (ANOVA, Bartlett's test and Scheffe's test) was applied for multiple comparisons with one control group (Tables 5–7). Four-fold contingency tables (Geigy, 1969) were used to evaluate the results of Fig. 3. $P < 0.05$ was the threshold for the verdict of statistical significance.

Drugs

All drugs were dissolved in saline and subcutaneously injected in a volume of 0.1 ml/10 g body weight. Caerulein diethylammonium hydrate (ceruletide) and its analogues were about 95% pure; they were made by Farmitalia Carlo Erba and kindly provided by Prof. Roberto de Castiglione, Milan; CCK-8 (90% pure) and L-prolyl-L-leucylglycine amide (MIF) were purchased from Bachem (CH-4416 Bubendorf/Switzerland). Other drugs used were harmine·HCl (Fluka), ibogaine·HCl (Serva), oxotremorine sesquifumarate (Serva), atropine H_2SO_4 (commercial), haloperidol (Janssen), biperiden·HCl (Knoll), ethopropazine·HCl (Bayer), methixene·HCl (Sandoz), trihexyphenidyl·HCl (Lederle) and clonazepam (Hoffmann-La Roche).

RESULTS

Dose–response relationships

The treatment of the mice with either harmine, ibogaine or oxotremorine (preceded by a subcuta-

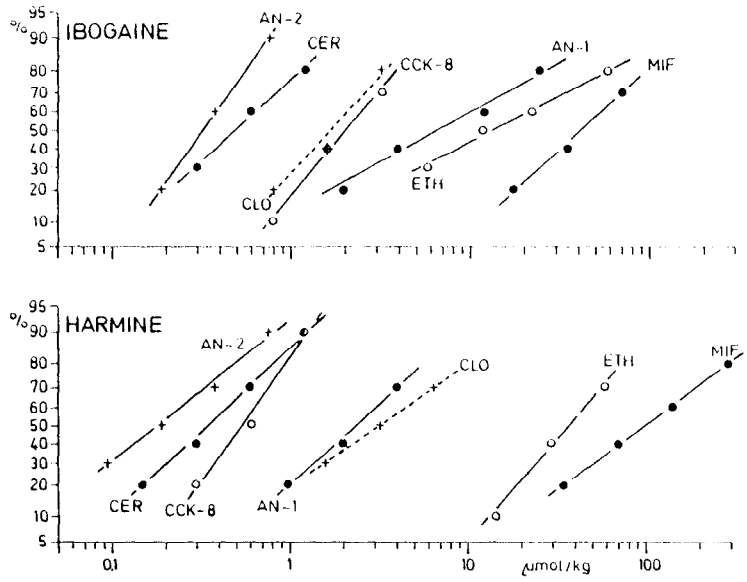

Fig. 2. Dose–response curves of selected compounds for the antagonism of the tremor induced by either harmine (5 mg/kg, lower part) or ibogaine (20 mg/kg, upper part). Abscissae: subcutaneously administered doses (μmol/kg, 10 min before tremorogen). Ordinates: percentage of mice showing a tremorolytic effect ($n = 10$ per dose). Regression lines were drawn on probability paper. Drugs: CER, ceruletide; CCK-8, cholecystokinin octapeptide; AN-1, analogue No. 1 (desulphated CER); AN-2, analogue No. 2 (Nle^8-CER); MIG (prolyl-leucylglycine amide); CLO, clonazepam; ETH, ethopropazine.

neous injection of saline, to be replaced by a tremorolytic compound) elicited tremors that were present in 100% of the mice 5, 10 and 15 min later (Fig. 1). This protocol allowed for the time-course of the central actions of the CCK-like peptides as shown in previous studies (cf. Introduction; the peptidic effects, following subcutaneous administration to mice, occurred within 5 min and reached a maximum about 10 min later). Reduction of the tremorogenic dose by 50% lessened the effect of harmine more than that of ibogaine (Fig. 1). This suggests that there are different dose–response relationships for both alkaloids. The results shown in Fig. 1 are in agreement with the observation that (in mice, after subcutaneous administration) the half-life both of the tremor and the concentration in the brain was longer for ibogaine than for harmine (Zetler *et al.*, 1972). Hence, with some reservation, the tremorogenic doses of harmine and ibogaine were considered to be equipotent, as will be discussed.

The tremorolytic effects were dose-dependent, which resulted in straight dose–response lines on probability paper (examples are shown in Fig. 2). Exceptions occurred with MIF when tested against the oxotremorine-induced tremor and with some other compounds when administered 30 min before either harmine or ibogaine (see text). All other experiments on tremor resulted in ED_{50} values expressing the tremorolytic potency.

Harmine-induced tremor

Cholecystokinin octapeptide, ceruletide, its analogues and MIF exerted tremorolytic effects (Table 1). Out of 12 peptides, only 5 were clearly (i.e. to a statistically significant extent) less active than ceruletide, namely (in decreasing order of potency) analogues Nos 4, 1, 6, 10 and MIF. The peptide Nle^8-CER had about twice the potency of ceruletide, however, the difference between the ED_{50}s of both peptides was not statistically significant. Of the analogues not differing in potency from ceruletide ($P > 0.05$), Nos 3, 5, 7, 9 and 11 were significantly less potent than No. 2. Hence, these compounds, together with analogue No. 2 and ceruletide may be considered to be a group of potent tremorolytic peptides of which Nle^8-CER had the greatest activity. The weakest tremorolytic effect was produced by MIF which was less potent than Nle^8-CER, ceruletide, CCK-8 and (the least active) analogue No. 10 by a factor of 533, 267, 178 and 7, respectively.

In separate experiments, the ED_{50} of ceruletide was administered *after* the tremorogenic effect of harmine had developed (Fig. 3). This dose of ceruletide, and also one quarter of it, reduced the tremor within a few minutes. This suggests that the tremorolytic effect of the peptides (given 10 min *before* harmine) was not an artifact arising from an interference with the absorption of harmine. The short latency is in agreement with a very rapid onset (2–5 min) of antinociception and ptosis after subcutaneous administration to mice and rats (Zetler, 1980a; Brasch and Zetler, 1982).

Ibogaine-induced tremor

The tremorolytic ED_{50}s (Table 1) were of the same order of magnitude as those against the harmine-induced tremor. Some peptides were less active

Table 1. Tremorolytic potency of ceruletide, its analogues and CCK-8, and of L-prolyl-L-leucylglycine amide (MIF), when administered (s.c.) 10 min before either harmine or ibogaine. The potency is expressed as mean effective dose (ED_{50} and 95% confidence range)

	Harmine-induced tremor			Ibogaine-induced tremor		
Analogue compound No.	ED_{50} (μmol/kg)	Slope function	RP	ED_{50} (μmol/kg)	Slope function	RP
Ceruletide (CER)	0.36 (0.22–0.59)	2.70	100	0.48 (0.29–0.80)	2.74	100
1. Desulphated CER	2.4* (1.39–3.99)	2.83	15	6.5* (3.19–13.31)	5.10	7
2. Nle^8-CER	0.18 (0.10–0.33)	3.23	200	0.32 (0.21–0.48)	1.91	150
3. Val^5, Nle^8-CER	0.67 (0.47–0.95)	2.02	54	0.61 (0.37–1.02)	3.22	79
4. $Met(O)^8$-CER	0.88* (0.53–1.46)	2.73	41	0.46 (0.29–0.74)	2.15	104
5. (β-$Asp)^9$-CER	0.55 (0.29–1.01)	4.08	66	1.48* (0.98–2.23)	2.26	32
6. $Phe(OH)^{10}$-CER	3.92* (2.62–5.87)	2.22	9	8.6* (5.74–12.79)	1.91	6
7. Boc-Leu^8-CER-(4–10)	0.42 (0.25–0.70)	2.73	86	0.79 (0.49–1.27)	2.59	61
8. Boc-Nle^8-CER-(4–10)	0.35 (0.21–0.60)	2.85	103	0.47 (0.24–0.91)	3.70	102
9. Nle^8-CER-(4–10)	0.41 (0.25–0.58)	2.75	88	0.51 (0.34–0.77)	2.28	94
10. $Tyr(SO_3H)^6$-CER-(6–10)	13.1* (8.33–20.6)	2.45	3	9.5* (5.63–16.11)	2.83	5
11. CCK-8	0.54 (0.36–0.81)	1.93	67	2.0* (1.23–3.21)	2.16	24
MIF	96.0* (55.8–163.8)	3.42	0.4	41.0* (25.1–56.9)	2.64	1

CER: Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2; note that analogues Nos 7–9 are heptapeptides, and No. 10 is a pentapeptide. Slope function = $(ED_{84}/ED_{50} + ED_{50}/ED_{16})/2$.

*Difference from the ED_{50} of CER is statistically significant.

RP: relative potency (CER = 100) rounded to the nearest integer (underlined if significant).

against ibogaine than against harmine, which led to statistically significant increases in the ED_{50} of analogues Nos 1, 5, 6 and CCK-8. When compared with ceruletide, analogues Nos 1, 5, 6 and 10 as well as CCK-8 and MIF had less potency. This agrees with the results on harmine except for analogue No. 5 and CCK-8 both of which did not differ from ceruletide in potency against harmine. Analogue No. 4 was a little more active against ibogaine than against harmine ($P > 0.05$) and thus of the same potency as ceruletide. In contrast to ceruletide and its analogues, MIF was twice as active against ibogaine ($P < 0.05$) as against harmine. Hence, the quantitative relationships between important peptides were not the same as with the harmine-induced tremor, however, MIF was still the least potent peptide differing in ED_{50} from Nle^8-CER, ceruletide, CCK-8 and analogue No. 10 by a factor of 128, 85, 21 and 4, respectively.

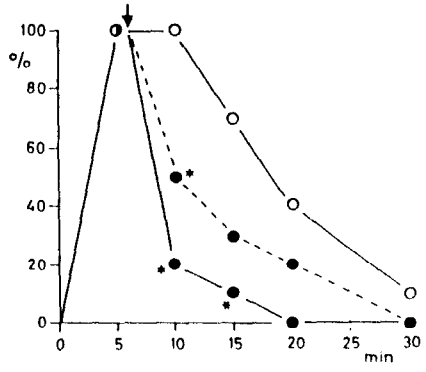

Fig. 3. Time-course of harmine-induced tremor when either saline (○——○), ceruletide (0.36 μmol/kg, s.c.; ●——●) or ceruletide (0.09 μmol/kg, s.c., ●----●) was administered (arrow) 6 min after harmine (5 mg/kg, s.c.). Ten mice per treatment. Abscissa: time following administration of harmine. Ordinate: percentage of animals showing tremor. Note that in each group 100% of the animals had tremor before the injection of either saline or ceruletide. *$P < 0.05$ for the difference from the corresponding control value (saline).

The CCK-like peptides produced ptosis (as was expected, see Table 6) that was present at the time when the tremorogens were administered. With respect to the central stimulatory nature of harmine and ibogaine, it is a notable secondary observation that the ptosis (even that following doses of peptide having only a weak tremorolytic effect) was not reduced or abolished by these tremorogens.

Effects of reference drugs against harmine and ibogaine

As can be seen from Table 2, all reference drugs were less potent than ceruletide and most of its analogues against the tremors induced by either

Table 2. Tremorolytic potency of reference drugs, when administered (s.c.) 10 min before either harmine, ibogaine or oxotremorine (for details see Table 1)

	Harmine			Ibogaine			Oxotremorine	
Drug	ED_{50} (μmol/kg)	Slope function	RP	ED_{50} (μmol/kg)	Slope function	RP	ED_{50} (μmol/kg)	Slope function
Atropine	5.2 (3.08–8.71)	2.80	7	3.0 (2.02–4.52)	2.21	16	0.76* (0.52–1.12)	2.12
Haloperidol	3.5 (2.43–4.91)	1.76	10	4.2 (2.80–6.31)	2.24	11	1.36* (0.83–2.21)	2.63
Biperiden	30.5 (18.2–50.9)	2.76	1	44.5 (20.89–95.01)	4.47	1	1.40* (0.95–2.17)	2.27
Ethopropazine	36.1 (22.4–58.3)	2.17	1	13.2* (6.07–28.65)	5.88	4	1.89* (1.25–2.86)	2.26
Methixene	57.8 (36.4–91.9)	1.70	0.6	9.5* (5.67–16.05)	3.28	5	1.55* (1.05–2.29)	2.17
Trihexyphenidyl	91.7 (67.2–125.2)	1.65	0.4	272.0* (209–354)	1.53	0.2	0.89* (0.72–1.09)	1.39
Clonazepam	3.2 (1.63–6.16)	3.73	11	1.68 (1.11–2.55)	2.28	29	1.43 (0.86–2.38)	2.75

*Difference from the ED_{50} against harmine is statistically significant.

harmine or ibogaine (Table 1). This applies especially to the anti-Parkinsonian drugs, from biperiden to trihexyphenidyl. The ibogaine-induced tremor had for atropine, haloperidol, biperiden and clonazepam, the same sensitivity as the harmine-induced tremor, whereas it was more resistant to trihexyphenidyl and more sensitive to both ethopropazine and methixene.

Oxotremorine-induced tremor

As was expected, all reference drugs with anticholinergic properties antagonized this type of tremor in a dose-dependent manner, which permitted the determination of ED_{50} values (Table 3). It was noted that the anti-Parkinsonian drugs (biperiden, ethopropazine, methixene and trihexyphenidyl) were many times more potent against oxotremorine than against the two indole alkaloids, whereas such differences were only small with haloperidol and even absent with clonazepam. On the other hand, ceruletide (0.3, 1.2 and 3.6 μmol/kg) was completely inactive and Nle^8-CER (1.5 and 6 μmol/kg) protected only 30 and 20%, respectively, of the animals (other ceruletide analogues not tested). This suggested that the tremorolytic effect of CCK-like peptides does not comprise the oxotremorine-induced tremor. At doses of 34, 68, 102, 136 and 272 μmol/kg MIF protected 10, 50, 30, 30 and 20%, respectively, of the animals. Hence, this peptide exerted only a weak effect that was not dose-dependent.

The time till testing

It was not clear how the time between the tremorolytic treatment and the injection of the tremorogen would influence the result. It seemed possible that 10 min was too short a period to allow the non-peptide tremorolytics to reach full efficiency. Therefore, the tremorolytic ED_{50} against harmine and ibogaine was determined for 8 compounds adminis-

Table 3. Influence of time on the tremorolytic potency of selected compounds

	Harmine-induced tremor			Ibogaine-induced tremor		
Treatment (s.c.)	ED_{50} (μmol/kg)	Significance*	Factor*	ED_{50} (μmol/kg)	Significance*	Factor*
Ceruletide (CER)	1.66 (0.96–2.88)	+	0.2	1.85 (1.17–2.92)	+	0.3
Nle^8-CER	0.31 (0.18–0.51)	0	0.6	1.07 (0.71–1.62)	+	0.3
Boc-Nle^8-CER-(4–10)	1.03 (0.57–1.86)	+	0.3	1.10 (0.58–1.76)	+	0.4
Nle^8-CER-(4–10)	1.23 (0.75–2.01)	+	0.3	1.26 (0.76–2.08)	+	0.4
Haloperidol	2.66 (1.46–4.85)	0	1.3	2.23 (1.14–4.34)	0	1.9
Methixene	43.0 (28.8–65.3)	0	1.3	Excitement (see text)		
Trihexyphenidyl	207 (125–341)	+	0.4	222 (146–338)	0	1.2
Clonazepam	4.53 (2.91–7.04)	0	0.7	6.02 (3.11–11.66)	+	0.3

*Statistically significant difference from the ED_{50} (10 min, shown in Tables 1 and 2) present (+) or absent (0). The factor results from ED_{50}, 10 min/ED_{50}, 30 min.
In these experiments, a tremorogen was administered 30 min after treatment.

Table 4. Potency of ceruletide and 4 non-peptidic reference drugs for the inhibition of exploratory rearing activity, and for the production of palpebral ptosis

Drug	Inhibition of rearing ED_{50} (and 95%-confidence range) (μmol/kg, s.c.)	Slope function	Ptosis
Ceruletide	0.015 (0.012 0.023)	1.54	Present*
Haloperidol	0.44 (0.25 0.77)	3.38	Absent
Clonazepam	1.3 (0.91 1.86)	2.16	Absent
Methixene	56 (45.6 69.6)	1.45	Present*
Trihexyphenidyl	101 (66.6 151.9)	2.07	Absent

*ED_{50} (μmol/kg, s.c.) for the production of ptosis: ceruletide, 0.012 (0.0097 0.016); methixene, 108 (74.6 157.6).

tered 30 min before the tremorogens. The results, presented in Table 3, show that none of the non-peptide drugs increased in potency when the waiting time was 30 min (there was only a tendency with haloperidol versus ibogaine). Depending on the type of tremor, losses in potency occurred after 30 min with trihexyphenidyl and clonazepam. This suggests that the waiting time of 10 min was appropriate. The potency of the peptides was mostly decreased after 30 min. Nevertheless, it is obvious that ceruletide and its analogues still had ED_{50}s less than those of methixene, trihexyphenidyl and clonazepam. Interestingly, the combination of methixene and ibogaine produced signs of central excitation (running, jumping, Straub's tail) that made tremor testing impossible.

No dependable dose response relationships were obtained with MIF (30 min) of which 20, 40, 60 and 80 mg/kg was tested against harmine and 40, 80, 160 and 320 mg/kg against ibogaine ($n = 10$/dose). The strongest tremorolytic effect of MIF was 60% protection against harmine (MIF, 40 mg/kg) and 50% protection against ibogaine (MIF, 320 mg/kg). Obviously, MIF had lost most of its activity 30 min after administration (approximate ED_{50} values against harmine, 109 μmol/kg; against ibogaine, 1092 μmol/kg).

These results suggest that the differences in potency shown in Tables 1 and 2 were not artifacts arising from the protocol which possibly favoured 1 compound more than another. It is also clear that the tremorolytic action of MIF was shorter lasting than that of ceruletide and those of its analogues that were tested 30 min after administration.

Sedative effects of reference drugs

Whether, or to what extent the central depressant actions of the CCK-like peptides are essential for the tremorolytic effects will be discussed. Therefore, it was important to learn if the reference drugs had any sedative potency. This was studied for inhibition of rearing and production of ptosis. Rearing was dose-dependently inhibited by haloperidol, clonazepam, methixene and trihexyphenidyl (Table 4). On a molar basis, these drugs were much less active than ceruletide. In the dose range used to determine the ED_{50} only ceruletide and methixene produced ptosis, however, the ED_{50} for methixene was 9000 times larger than that of ceruletide. Neither inhibition of rearing nor ptosis was seen after administration of the following drugs ($n = 12$ per dose): biperiden (5, 10, 20 and 40 mg/kg, s.c.); ethopropazine (8 and 20 mg/kg, s.c.); atropine (2 and 5 mg/kg, s.c.); MIF (20, 80 and 320 mg/kg, s.c.). Biperiden even enhanced the rearing activity at all doses used.

Tremor and body temperature

The CCK-like peptides produce hypothermia in mice and ceruletide is most potent in this respect (Zetler, 1982b). Table 5 refers to the question of whether the hypothermic and the tremorolytic effect may be connected (only a few relevant examples are shown in this table). When the pretreatment was saline, both harmine and ibogaine were hypothermic, but ibogaine much less so in spite of the equality in tremorogenic effect. Ceruletide, $(\beta\text{-Asp})^7$-CER and CCK-8 augmented these decreases in temperature (note the differences in strength of the effect), but $Met(O)^8$-CER was very weak in this respect; Nle^8-CER-(4–10), however, abolished the harmine-induced hypothermia, but augmented (at the same dose) the hypothermic effect of ibogaine. Differences between the thermoregulatory effects of the two tremorogens were also seen with MIF and clonazepam: the former was inactive against harmine and antagonized ibogaine, whereas the latter did not alter the effect of ibogaine but increased that of harmine. These differences between treatments, together with the tremorolytic equieffectiveness of the same treatments suggest an independence between antagonism of tremor and lowering of body temperature.

DISCUSSION

This study demonstrates that CCK-8, ceruletide and analogues of ceruletide antagonized dose-dependently the tremors caused by both harmine and ibogaine, but not the oxotremorine-induced tremor. This was parallelled by MIF, for which (as in present experiments) only doubtful tremorolytic effects against oxotremorine have been described (Plotnikoff, Kastin, Anderson and Schally, 1972; Castensson, Sievertsson, Lindeke and Sum, 1974;

Kruse, 1977; Björkman, Lewander and Zetterström, 1980; Dickinson, Slater and Longman, 1981). It must therefore be stressed that the antagonism of harmine and ibogaine by MIF (demonstrated for the first time) was clear-cut, dose-dependent and reproducible. All the CCK-like peptides were far more potent than MIF against the indole alkaloids, and many of them were also superior to the non-peptide reference compounds.

Haloperidol and the anti-Parkinsonian drugs were 6–100 times more potent against oxotremorine than against harmine, and this may be explained by their central anticholinergic mechanism of action. Nevertheless, the ED_{50} values of these drugs against oxotremorine were mostly above 1 μmol/kg, and hence larger than those of many CCK-like peptides against the indole alkaloids. This stresses the potency of the peptides.

The tremor induced by ibogaine had features different from those of the harmine-induced tremor. Many CCK-like peptides, although not all of them, were less effective against ibogaine than against harmine. In contrast, MIF was twice as potent against ibogaine than against harmine, and this is similar to ethopropazine and methixene that were 3 and 30 times, respectively, more active against ibogaine than against harmine. Hence, the 2 indole alkaloids were useful to differentiate the tremorolytic effects of MIF from those of CCK-like peptides. It may also be presumed that harmine and ibogaine produce tremor by different mechanisms. Therefore, it is important to note that iboga alkaloids, in contrast to harmine, antagonize the cataleptogenic effects of reserpine and prochlorperazine in mice, but do not inhibit monoamine oxidase (Zetler, 1964).

This discussion presupposes the 3 different tremorogens to produce (at the doses used) tremors of the same intensity. This cannot be taken for granted but is suggested by the finding that (a) the majority of peptides and non-peptidic drugs antagonized harmine and ibogaine at the same doses and (b) clonazepam was equipotent against all 3 tremorogens. However, the latter fact also suggests the tremorolytic effect of clonazepam to be least specific.

The many central effects of CCK-like peptides comprise those that can be considered depressant or sedative, i.e. ptosis-producing, rearing-inhibiting, cataleptogenic and hexobarbital-potentiating effects. It is not clear whether the tremorolytic actions under study were simple consequences of a general depression of central functions. To discuss this problem, it is very useful to consider, besides ceruletide and CCK-8 the 7 analogues of ceruletide for which (a) tremorolytic ED_{50}s exist and (b) the strength of the sedative action can be expressed as an ED_{50} as previously determined (Zetler, 1981d, 1982c). It was appropriate not to prefer one of the depressant effects, but to calculate for each effect an index expressing its strength relative to that of the tremorolytic effect, and to evaluate the resulting 36 indices

Table 5. Change in body temperature ($\bar{x} \pm SE$) during tremor caused by either harmine or ibogaine, and influence of equieffective tremorolytic treatments (10 mice/group)

		Harmine (5 mg/kg)					Ibogaine (20 mg/kg)				
Exp. No.	Treatment	Dose (mg/kg)	Mice with tremor (%)	Change in temperature (°C)	Difference from exp. No. 1	Difference from exp. No.	Dose (mg/kg)	Mice with tremor (%)	Change in temperature (°C)	Difference from exp. No. 1	Difference from exp. No.
1.	Saline		100	-2.1 ± 0.59				100	-0.9 ± 0.35		
2.	Ceruletide (CER)	0.8	10	-4.3 ± 0.39	b		1.6	20	-4.8 ± 0.38	b	
3.	Met(O)^4-CER	2	30	-2.9 ± 0.30	NS	2:b	1	30	-1.6 ± 0.21	NS	2:b
4.	(β-Asp)^4-CER	4	10	-3.7 ± 0.47	b	2:NS	4	20	-4.1 ± 0.50	b	2:NS
5.	Nle^4-CER-(4–10)	1	20	-0.5 ± 0.29	b		1	20	-1.7 ± 0.44	a	2:b
6.	CCK-8	1.35	10	-3.1 ± 0.50	a	2:b	3.6	30	-3.2 ± 0.42	b	2:b
7.	MIF	80	20	-1.8 ± 0.45	NS	2:b	20	30	-0.1 ± 0.22	a	8:NS
8.	Clonazepam	2	30	-3.4 ± 0.36	b	2:NS	1	20	-0.6 ± 0.42	NS	2:b

The results were obtained in the course of the experiments summarized in Tables 1 and 2. Note that the different drug treatments were nearly equitremorolytic (70–90% of the animals were protected from tremor).
The initial body temperature (before the injection of saline) was in exp. No. 1, 37.5 ± 0.88 C ($\bar{x} \pm SD$) with the harmine group and 37.5 ± 0.65°C with the ibogaine group.
NS, $P > 0.05$; a, $P < 0.05$; b, $P < 0.01$.

of sedative influence. Table 6 refers to the harmine-induced tremor and suggests a tremorolytic potency not running parallel with the capability to inhibit rearing activity, to produce ptosis, to elicit catalepsy and to prolong the hexobarbital-induced sleep. Compared with these 4 properties, the tremorolytic potency of ceruletide was less by a factor of 24, 30, 33 and 17, respectively. However, CCK-8 exerted depressant "side effects" which were many times less prominent than with ceruletide (ptosis being an exception). The geometric mean of the indices of sedative influence may express the strength of sedation relative to the tremorolytic effect. From this point of view, ceruletide and Val^5,Nle^8-CER were the least favourable compounds, whereas (β-Asp)^9-CER and Nle^8-CER-(4–10) were the peptides with the most selective tremorolytic effect.

When this evaluation was applied to the ibogaine-induced tremor (Table 7), it was again obvious that the tremorolytic effect was least selective with ceruletide and its analogues Nos 2 and 3, but much more prominent with the other analogues. However, a closer inspection of Tables 6 and 7 shows that the results varied with the type of tremor. Whereas the tremorolytic effect of Nle^8-CER-(4–10) was of equal distinctness, that of both (β-Asp)^9-CER and CCK-8 was more pronounced with harmine, but that of Met(O)^8-CER more so with ibogaine. Nevertheless, both Tables may suggest that the tremorolytic effect is not a simple result of a general depression of central nervous function. This view is stressed by the tremorolytic effects of desulphated ceruletide, Phe(OH)^{10}-CER and Tyr(SO$_3$H)^6-CER-(6–10), which were omitted from Tables 6 and 7 because they have been found in previous studies to exert only minimal, if any depressant effects.

Methixene was the sole reference drug that produced, like ceruletide, both inhibition of rearing and ptosis. However, this property did not provide methixene with more tremorolytic potency than ethopropazine (Table 2) that did not cause any sedation. Likewise, the tremorolytic effects of atropine, biperiden and MIF were not accompanied by reduced rearing and ptosis. On the other hand, haloperidol was not more tremorolytic than clonazepam in spite of its being more rearing-inhibiting than the benzodiazepine. Without going into further details, it seems to be clear that the tremorolytic effect of both the peptides and the non-peptidic reference drugs is not a simple consequence of a general central depression.

Hypothermia, too, can be excluded as a causative factor for the tremorolytic action of the peptides and the reference drugs. This suggests itself not only from the present results (Table 5) but also from a comparison of tremorolytic with hypothermic potency of analogues of ceruletide (Zetler, 1982b). It may suffice to mention that Nle^8-CER was more tremorolytic than ceruletide in spite of having only 22% of the hypothermic potency of ceruletide, or that Boc-Leu^8-CER-(4–10), Boc-Nle^8-CER-(4–10) and

Table 6. Appraisal of the importance of sedation for the tremorolytic effect. The index of sedative influence is a quotient of ED_{50} values and indicates how many times a given central depressant activity was more potent than that antagonizing the harmine tremor

No.	ED_{50} (nmol/kg)					Index of sedative influence					Statistics[a]		
	a HTR	b REA	c PTO	d CAT	e HEX	a/b	a/c	a/d	a/e	Geometric mean	Difference from CER	Difference from
CER	360	15	12	11	21	24	30	33	17	25.2		
2. Nle^8-CER	180	27	10	7	54	7	18	26	3	10.0	*	No. 3*
3. Val^5, Nle^8-CER	670	73	25	12	33	9	27	56	29	22.8	NS	
4. Met(O)^8-CER	880	292	504	73	585	3	2	12	1.5	3.1	**	
5. β-Asp)^9-CER	550	301	233	244	739	2	2	2	0.7	1.6	**	CCK-8*
7. Boc-Leu^8-CER-(4–10)	420	155	140	38	234	3	3	11	2	3.8	**	
8. Boc-Nle^8-CER-(4–10)	350	63	74	43	167	6	5	8	2	4.5	**	
9. Nle^8-CER-(4–10)	410	398	176	86	510	1	2	5	0.8	1.7	**	No. 2** No. 8**
CCK-8	450	350	50	184	181	1.5	11	3	3	3.5	**	

Abbreviations: HTR, harmine-induced tremor; REA, inhibition of rearing; PTO, ptosis; CAT, catalepsy; HEX, prolongation of hexobarbital-induced sleep. Values for HTR come from Table 1, the other figures from previous studies (Zetler, 1981d, 1982c).

[a]Statistics after log transformation: * $P < 0.05$; ** $P < 0.01$.

Table 7. Appraisal of the importance of sedation for the antagonism of the ibogaine-induced tremor. For details, see Table 6 (this applies also to the values of REA, PTO, CAT and HEX)

	Index of sedative influence						
						Statistics	
No.	a:b	a:c	a:d	a:e	Geometric mean	Difference from CER	Difference from
CER	32	40	44	23	33.7		
2. Nle^8-CER	12	32	46	6	18.0	NS	No. 3, NS
3. Val^5, Nle^8-CER	8	24	51	19	20.8	NS	
4. $Met(O)^5$-CER	2	0.9	6	0.8	1.7	**	No. 5*
5. (β-Asp)^5-CER	5	6	6	2	4.4	**	CCK-8**
7. Boc-Leu^5-CER-(4–10)	5	6	21	3	6.6	**	CCK-8, NS
8. Boc-Nle^8-CER-(4–10)	7.5	6	11	3	6.2	**	No. 2**
9. Nle^8-CER-(4–10)	1	3	6	1	2.1	**	No. 8** CCK-8**
CCK-8	6	40	11	11	13.1	*	

*$P < 0.05$; **$P < 0.01$.

Nle^8-CER-(4–10) were virtually as tremorolytic as ceruletide although having only 4 to 8% of the hypothermic potency.

The question of a relationship between the tremorolytic potency of CCK-like peptides and their antagonism of convulsions induced by both harman and picrotoxin (Zetler, 1981d, 1982c) is worth discussing in view of the idea (a) that harmaline may owe its tremorogenic activity to an interaction with the benzodiazepine receptor (Robertson, 1980) and (b) that CCK-like peptides may modify the function of the "GABA receptor regulator unit" (Zetler, 1980b, 1981a, c, 1982c); a further hint is the marked tremorolytic action of clonazepam which has very high affinity for the specific benzodiazepine receptors in rat and human brain (Braestrup and Squires, 1978; Möhler and Okada, 1978). In fact, tremorolytic potency was high with peptides having good anti-harman and anti-picrotoxin activity [CER; Nle^8-CER; Val^5,Nle^8-CER; Boc-Nle^8-CER-(4–10)] and vice versa [desulphated ceruletide; $Phe(OH)^{10}$-CER; $Tyr(SO_3H)^6$-CER-(6–10)]. However, Nle^8-CER-(4–10) is a notable exception because its tremorolytic activity paralleled that of ceruletide, whereas its harman- and picrotoxin-antagonistic activity was only 16 and 8%, respectively, that of ceruletide.

Ceruletide and haloperidol have been found to be equal in antistereotypic potency (model: methylphenidate-induced compulsatory gnawing in mice), whereas CCK-8 was less active by a factor of 3.3 (Zetler, 1981a). These relationships are not confirmed by the present results on harmine-induced tremor (peptides superior to haloperidol; no difference between ceruletide and CCK-8) nor by those on ibogaine-induced tremor (peptides and haloperidol equal in activity) or those with oxotremorine (only haloperidol active). Certainly, analogues of ceruletide having high tremorolytic potency [Nle^8-CER; Boc-Nle^8-CER-(4–10); Nle^8-CER-(4–10)] were also very active as antistereotypics, however, desulphated ceruletide and $Phe(OH)^{10}$-CER clearly were tremorolytic whilst they exerted no antistereotypic effect in doses up to 7.9 and 5.9 μmol/kg, respectively (Zetler, 1981d). It may be concluded that tremorolytic and antistereotypic actions are independent.

It follows from this discussion that CCK-like peptides have a tremorolytic activity that is not just a functional consequence of any other central effect discussed. The present results also demonstrate that CCK-like peptides have pharmacological properties which would enable them to play a role in the physiological regulation of involuntary motor activity (see Introduction). Indeed, cholecystokinin receptors that also bind caerulein (Hays, Beinfeld, Jensen, Goodwin and Paul, 1980) are decreased in basal ganglia and cerebral cortex of Huntington's chorea (Hays, Beinfeld, Jensen, Goodwin and Paul, 1981) and CCK-8 immunoreactivity levels are lower in the substantia nigra from Parkinsonian patients (Studler, Javoy-Agid, Cesselin, Legrand and Agid, 1982). Accordingly, CCK-like peptides might be useful in disorders such as tremors or intentional myoclonus. For this purpose the heptapeptide, Nle^8-CER-(4–10) would be a candidate, because its tremorolytic effect was least burdened with other central depressant effects (see Tables 6 and 7). However, side effects from both the intestinal tract and the circulation may be envisaged.

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