Analgesic activity of certain tripeptide-β-phenethylamide analogs

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Summary. Studies of the analgesic effect of tripeptide-β-phenethylamides are described, and their structure-activity relationship is discussed. SD-25, which has a methyl group at R_2, and a hydroxymethyl group at R_3 of β-phenethylamide, was the most potent one of the 8 analogs tested.

Since the demonstration by Hughes et al.[2] that pentapeptides, methionine-enkephalin (Met-Enk) and leucine-enkephalin (Leu-Enk) extracted from mammalian brain exhibit opioid agonist activity, many analogs of Enk have been synthesized, and the properties of their opioid activity have been investigated[3,4]. In this paper we describe the analgesic effect of certain tripeptide-β-phenethylamides structurally different from Enk (fig.).

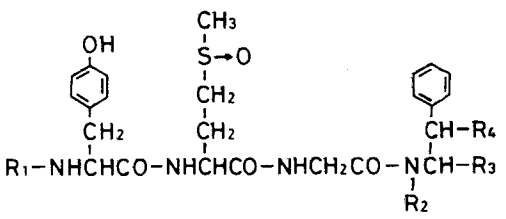
Materials and methods. Male ddY strain mice weighing 20–22 g were used. The analgesic activity was evaluated using a hot plate method and a writhing assay. The hot plate method was a modification of the method of Woolfe and MacDonald[5]. Response determined as the endpoint was either 1. licking or shaking of the hindpaw or 2. jumping out of the cylinder. Animals showing a reaction time of 3–8 sec in a control trial were selected. Tested compounds were dissolved in saline and injected s.c. For each dosage, at least 5 animals were used. The hot plate test was performed at 15, 30, 45, 60, 90, 120 and 180 min after drug administration; if an animal did not respond to heat stimulus within 30 sec, it was removed from the cylinder immediately to avoid excessive injury. From the percentage of animals showing a reaction time longer than 2 times the pre-drug value at the peak effect of each drug, an ED_{50} was calculated according to the method of Litchfield and Wilcoxon[6]. The hot plate test was performed under 'blind' conditions in order to avoid obserbers' bias. In the writhing assay, chemical pain was induced by i.p. injection of 0.1 ml of 0.6% acetic acid solution per 10 g b.wt after the method of Koster et al.[7], and thereafter, the number of writhes were counted for 30 min. Test compounds were given s.c. 20 min before the administration of the writhing agent. The ED_{50} of the test compounds was calculated from the percentages of animals showing less than half the number of writhes counted in the control group.

Results and discussion. The analgesic activity of SD-25 was much more potent than that of morphine, and those of SD-20, OE-30 and OE-32 were almost equipotent to, or slightly less effective than, that of morphine with both assay methods (table). OE-31 was more efficacious than pentazocine, but its activity is about a quarter of that of morphine. OE-33, OE-34 and OE-35 were more effective than pentazocine in suppressing the number of writhes, but those compounds were less effectual than pentazocine in the hot plate method. It is worthwhile to mention that all these compounds could produce long-lasting analgesia, reaching a maximum intensity about 40–50 min after injection; a significant effect persisted for 180 min. As the dose increased, the analgesic effect became more pronounced (dose-response relation). The analgesic effect of Met-Enk was very weak when administered by s.c. injection.

In the comparison of the analgesic effect of SD- and OE-compounds, it was found that SD-20, SD-25 and OE-30 were more effective than OE-33, OE-34 and OE-35, respectively. This means that the addition of an allyl group to the terminal amino group of tyrosine reduces analgesic effect. Pless et al.[8] and Roemer et al.[9] reported that the substitution of tyrosine in H-Tyr-D-Ala-Gly-MePhe-Met(O)-ol by various substituents such as H-Arg-Tyr, H-Glu-Tyr, Ac-Tyr, Me_2-Tyr and N-cyclobutylmethyl-L-Tyr resulted in weak analgesia, though methylation of tyrosine increased the analgesic activity. It was also found that the effectiveness of SD-25 and OE-34 was superior to that of SD-20 and OE-33, respectively, and the activity of OE-30 was greater than that of OE-31. This may indicate that the introduction of a methyl group at R_2 is beneficial for analgesia, but introduction at R_4 is not. Pless et al.[8] and Morley[10] reported that the methylation of phenylalanine in Enk analogs augments the analgesic potency. The finding that SD-25 and OE-34 are more potent than OE-30 and OE-35 suggests that the introduction of a hydroxymethyl group at R_3 enhances the analgesic activity. McGregor et al.[11] reported that the N-terminal tetrapeptide amide analog of Enk (H-Tyr-D-Ala-Gly-Phe-NH_2) is approximately equipotent to highly active pentapeptides. However, the replacement of phenylalanine by β-phenethylamide does not decrease analgesic potency as is shown in the present experiment.

ED_{50}-values of the analgesic effects of some tripeptide-β-phenethylamides

Drugs	ED_{50} (mg/kg, s.c., 95% confidence limits) Hot plate method	Writhing method
Morphine	3.20 (1.70–6.10)	0.24 (0.18–0.32)
Pentazocine	30.0 (16.9–53.4)	5.08 (4.03–8.35)
Met-Enk	> 200.0	49.5 (22.3–109.9)
SD-20	4.80 (2.50–9.10)	0.06 (0.04–0.08)
SD-25	0.60 (0.40–1.10)	0.014 (0.006–0.031)
OE-30	5.20 (2.74–9.88)	0.96 (0.46–2.02)
OE-31	14.0 (8.75–22.4)	1.40 (0.78–2.52)
OE-32	4.70 (2.04–10.8)	0.82 (0.29–2.30)
OE-33	110.0 (68.3–177.1)	3.50 (2.30–5.32)
OE-34	52.0 (35.1–77.0)	2.60 (1.55–4.37)
OE-35	74.0 (47.7–114.7)	3.20 (1.45–7.48)



Compounds	R_1	R_2	R_3	R_4
SD-20	H	H	CH_2OH	H
SD-25	H	CH_3	CH_2OH	H
OE-30	H	CH_3	H	H
OE-31	H	CH_3	H	CH_3
OE-32	H	$CH(CH_3)_2$	H	H
OE-33	CH_2=CH–CH_2	H	CH_2OH	H
OE-34	CH_2=CH–CH_2	CH_3	CH_2OH	H
OE-35	CH_2=CH–CH_2	CH_3	H	H

Chemical structures of some tripeptide-β-phenethylamide analogs.

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Positive inotropic and chronotropic effect of alloimmune sera on isolated mouse atria[1]

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Summary. The effects of alloimmune sera on the contractile tension and frequency of spontaneously beating isolated mouse atria were explored. Immune sera enhanced frequency as well as tension; both effects were blocked by the presence of propranolol. In contrast, pretreatment with 6-OH dopamine potentiated the stimulatory action of immune sera.

In previous work it was documented that the sera of chagasic patients containing an antibody (EVI antibody) can interact with the plasma membrane, inducing functional changes in isolated rat atrial preparations[2]. The EVI positive human chagasic sera (EVI(+)S) could influence post-synaptic beta adrenoceptor sites of the plasma membrane acting as a partial beta agonist, increasing tension and frequency. Furthermore, EVI(+)S diminished the reactivity to exogenous norepinephrine through a reversible augmentation of its extraneuronal uptake[2,3]. In addition we have observed that EVI(+)S modified the action of ouabain on cardiac tissue through an activation of the beta adrenoceptors, increasing the influx of calcium[4,5].

In order to investigate whether sera directed againts other antigens could have similar effects on myocardial contractions, a series of experiments using alloimmune sera were done. We show here that BALB/c anti CF1 mouse sera are able to modify the physiological behaviour of isolated CF1 mouse atria. To determine the nature of the action of the alloimmune sera, the effects of propranolol and 6-hydroxydopamine were also studied.

Methods. Animals. Young adult (2-4 months) inbread BALB/c and CF1 mice from our colony were used throughout.

Sera. Immunizations were done with pooled lymphoid cells (from spleen, lymph nodes and thymus) obtained by pressing the organs through a stainless steel mesh. Cells were suspended in phosphate buffered isotonic solution (PBS). All immunizations were carried out between animals of the same sex. BALB/c mice were immunized by 1 i.d. injection of 10^7 CF1 lymphoid cells followed by 1-5 boosters of 3×10^7 CF1 lymphoid cells i.p. at weekly intervals. Animals

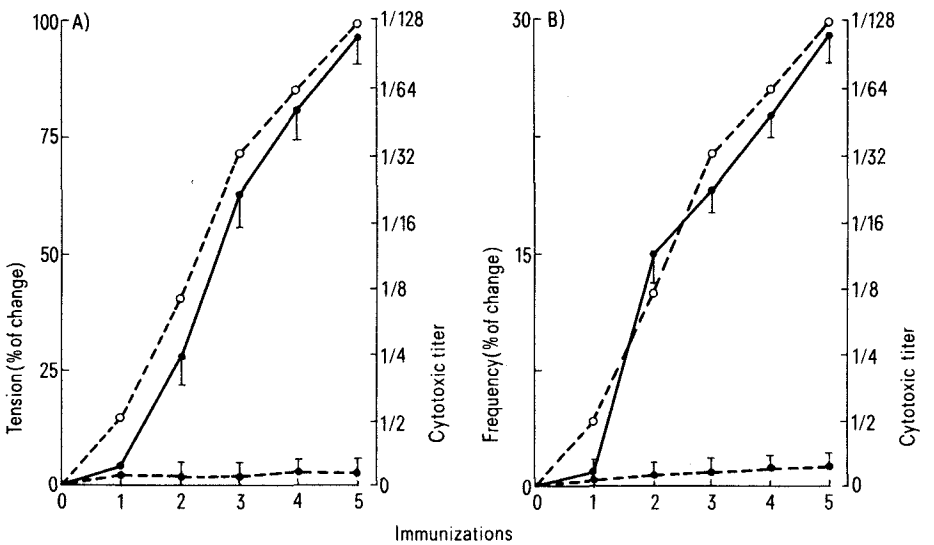

Figure 1. Positive and chronotropic effect of alloimmune sera on spontaneously beating atria. Cytotoxic titers (O - - - O) are shown in A and B in relation with the number of immunization. *A* Tension and *B* frequency in presence (● - - - ●) and absence of propranolol (●——●) are expressed as percent change from initial control ± SEM (n = 8 in each group).