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PRELIMINARY COMMUNICATIONS

ELEVATION OF ARTERIAL BLOOD PRESSURE IN CONSCIOUS DOGS BY DES-TYR^1-D-ALA^2-LEUCINE-ENKEPHALINAMIDE

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We have demonstrated that intravenously administered leucine-enkephalin (LE) increases heart rate (HR), respiratory rate (RR), and systolic (CA_s), diastolic (CA_d), and mean (CA_m) systemic arterial blood pressures (SBP) in conscious, chronically instrumented dogs (1). Enkephalins also increase SBP in unanesthetized rats (2). Naloxone inhibits the increases in both HR and SBP, suggesting that these responses are mediated by opioid receptors (2,3).

Opioid receptor binding, *in vitro* tissue activity, and antinociceptive properties have been used to assess the structure-activity relationships of enkephalin analogs. Substitution of D-alanine for L-glycine in the second position has resulted in greater biological activity (4). Although the essential structural feature required for *in vitro* activity and receptor binding is the aromatic hydroxyl moiety of L-tyrosine (5,6), Des-Tyr^1 enkephalin analogs have been utilized to inhibit the enkephalinase activity (7).

We observed, in our initial attempts to potentiate the activity of LE by enkephalinase inhibition with the LE analog Des-Tyr^1-D-Ala^2-Leu^5 enkephalinamide (DTALE), that DTALE has vasopressor activity by itself which is not inhibited by naloxone (2 mg/kg). This indicates that the N-terminal tyrosine is not essential for *in vivo* biological activity.

MATERIALS AND METHODS

Microfilaria-free mongrel dogs were chronically instrumented using the methodology described previously (1); LE was obtained from Calbiochem-Behring (LaJolla, CA) and DTALE from the Sigma Chemical Co. (St. Louis, MO). Naloxone was supplied by Endo Laboratories (Wilmington, DE). LE and DTALE were dissolved in 0.01 M acetic acid in 0.9% saline at a concentration of 1 mg/kg, and a dose of 35 μg/kg was administered as an intravenous bolus. Naloxone was administered at a dose of 2 mg/kg intravenously, and subsequent LE or DTALE was given 10 min later. Each experiment was performed once in each dog.

RESULTS AND DISCUSSION

In conscious, chronically instrumented dogs, LE increases HR, RR, and SBP in a dose-dependent fashion. The simultaneous elevation of HR and SBP indicates suppression of baroreceptor reflexes (1,2). Preliminary experiments in our laboratory have demonstrated that

D-Ala^2-Leu^5 enkephalinamide (DALE) is qualitatively similar to LE. In contrast, DTALE (35 μg/kg, i.v.) exerted a pressor effect (Table 1), but with no increase in RR and a reduction in HR, the latter probably reflecting a baroreceptor-mediated increase in vagal tone. The time sequence of this pressor response was similar to that of LE, beginning within 20 sec after administration and generally terminating by 180 sec. Naloxone (2 mg/kg, i.v.) did not alter this pressor response to DTALE.

Table 1. Cardiopulmonary responses to DTALE in the conscious dog*

	HR	RR	CA_s	CA_d	CA_m
Before naloxone					
Baseline	115 ± 12	18 ± 1	140 ± 8	69 ± 6	103 ± 6
Maximum	102 ± 13	20 ± 1	168 ± 6†	97 ± 7†	130 ± 8†
After naloxone					
Baseline	111 ± 19	19 ± 5	128 ± 6	75 ± 6	107 ± 7
Maximum	99 ± 18	18 ± 3	178 ± 8†	114 ± 6†	141 ± 9†

*Values are mean ± S.E.M. (N = 8 for pharmacological responses to DTALE and N = 4 for post-naloxone responses). Statistical analyses were performed using Student's t-test; a dagger indicates $P < 0.05$. Heart rate is expressed in beats/min, respiratory rate in cycles/min, and systolic, diastolic, and mean systemic arterial pressure in mm Hg.

Both LE and DALE increase HR, RR, and SBP, decrease baroreceptor sensitivity, and are inhibited by naloxone and, therefore, are opioid receptor agonists. DTALE elicited an increase in BP but did not appear to influence the baroreceptor mechanism; the failure of naloxone to inhibit this pressor response suggests that it is not mediated through opioid receptors, at least as they are commonly conceived. Thus, although the N-terminal tyrosine residue is required for the full naloxone-sensitive enkephalin response, it is not essential for all biological activity. Furthermore, the pressor responses to LE (or DALE) and DTALE appear to be mediated via different mechanisms which may be differentiated on the basis of naloxone sensitivity.

There is currently considerable interest in the role of opioid peptides in BP control. It now appears that naloxone-insensitive pathways and enkephalin analogs lacking the N-terminal tyrosine residue may contribute to BP control and thus deserve more extensive study.

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