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LEUCINE ENKEPHALIN ANTAGONIZES NOREPINEPHRINE-INDUCED $^{45}Ca^{++}$ ACCUMULATION IN RAT ATRIA

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Exposure of rat atrial slices to 10^{-5} M norepinephrine (NE) for 10 minutes increases $^{45}Ca^{++}$ accumulation from 1.64 ± 0.10 to 2.23 ± 0.06 nmol/mg tissue. In the presence of leucine enkephalin (10^{-8} M), NE-stimulated $^{45}Ca^{++}$ uptake is reduced to 1.44 ± 0.10 nmol/mg tissue. The effect of leu-enkephalin is reversed in the presence of 10^{-7} M naloxone, NE-stimulated $^{45}Ca^{++}$ uptake being increased to 2.17 ± 0.15 nmol/mg tissue. The results support a direct interaction of leu-enkephalin with β-agonist-stimulated Ca^{++} flux in rat atria, and correlate with the previously reported enkephalin antagonism of NE-induced positive chronotropy in the same tissue.

The enkephalin opiate peptides are widely distributed in the brain and periphery of numerous species, where they may play a role in cardiovascular regulation on both peripheral and central levels (1). In this regard, we have recently reported (2) that enkephalin peptides significantly attenuate the positive chronotropic response of isolated, spontaneously beating rat atria to (-)-norepinephrine (NE). The attenuation is both specific for adrenergic receptor mediated chronotropy, and is dependent upon the presence of external Ca^{++} (3). In view of these findings, and the recent suggestion that opiate peptides may restrict Ca^{++} flux in effector tissues (4,5), we have examined the effect of leucine enkephalin on NE-induced $^{45}Ca^{++}$ accumulation in slices of rat atria. We report here that leu-enkephalin produces a naloxone-reversible antagonism of NE-induced $^{45}Ca^{++}$ accumulation in this tissue.

MATERIALS AND METHODS

Sprague-Dawley rats of either sex (150-200 g) were decapitated, and the hearts were quickly removed and placed in an oxygenated tris buffer (NaCl 140, KCl 5, $CaCl_2$ 1.5, $MgSO_4$ 1.2, tris 10, glucose 10, ascorbic acid 0.1, Na_2EDTA 0.05 mM; pH 7.4)(6). The atria were quickly dissected, and opened to provide two strips weighing 10-15 mg each. The strips were mounted on fine wire hooks constructed of 20 gauge monel as previously described (6). The mounted strips were incubated at 37° with aeration (100% O_2) for 10 min, then were transferred to fresh medium containing tracer amounts of $^{45}CaCl_2$ (1 μ Ci/ml). (-)-NE bitartrate

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(10^{-5} M), naloxone HCl (10^{-7} M) and leucine enkephalin (10^{-8} M) as desired were also present in this medium. At 5 min intervals, tissue samples were removed, immersed briefly in cold (4°) isotonic EGTA (2 mM, pH 7.0) to remove superficially bound Ca^{++}. The tissue samples were then blotted, weighed, and homogenized in ethanol. $^{45}Ca^{++}$ accumulation was determined by liquid scintillation counting (Atomlight, New England Nuclear). All samples were corrected for quenching. $^{45}Ca^{++}$ accumulation was expressed as nmol $^{45}Ca^{++}$/mg tissue/time interval.

Sprague-Dawley-derived rats were obtained from Biological Research Labs, Arvada, CO. Leucine enkephalin was obtained from Peninsula Labs, Belmont, CA. (-)-Norepinephrine bitartrate and naloxone HCl were obtained from Sigma Chemical Co., St. Louis, MO. $^{45}CaCl_2$ (4-50 Ci/g calcium) was obtained from New England Nuclear Corp., Boston, MA.

RESULTS

The time course of accumulation of $^{45}Ca^{++}$ by control tissue and tissue stimulated with 10^{-5} M NE is shown in Figure 1. Stimulation of $^{45}Ca^{++}$ uptake is maximal (36%) and quite significant at 10 min of incubation, the levels of accumulation being elevated from 1.64 ± 0.10 to 2.23 ± 0.06 nmol/mg tissue. Control and NE-stimulated $^{45}Ca^{++}$ accumulation reach the same value at 15 min, reflecting equilibration of tracer.

The effect of 10^{-8} M leucine enkephalin and naloxone on NE-stimulated $^{45}Ca^{++}$ accumulation at 10 min is shown in Figure 2. 10^{-8} M leucine enkephalin completely antagonized the NE-stimulated uptake of $^{45}Ca^{++}$, reducing accumulation to control

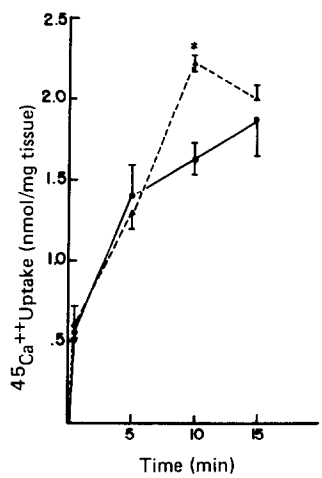

Figure 1. The time course of $^{45}Ca^{++}$ accumulation by rat atrial strips. Pieces of rat atria (10-15 mg) were incubated with $^{45}Ca^{++}$ in the presence (▲) and absence (●) of 10^{-5} M NE. At 5 min intervals, samples were removed, rinsed with 1 mM EGTA, blotted, weighed and homogenized in ethanol. $^{45}Ca^{++}$ uptake was determined by scintillation counting. Mean ± S.E.M. of 6-12 determintations.

*significantly different from control at $p<0.005$.

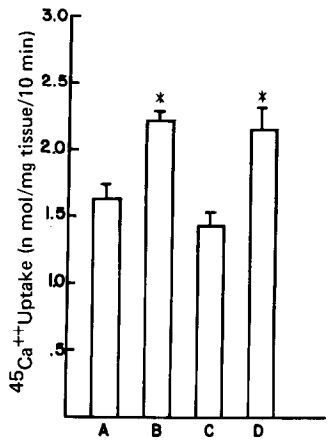

Figure 2. The effect of enkephalin on the 10 min accumulation of $^{45}Ca^{++}$ by rat atrial strips. The experimental conditions were the same as described in the legend of Figure 1. A: control, B: 10^{-5} M NE, C: 10^{-5} M NE and 10^{-8} M leu-enkephalin, and D: 10^{-5} M NE, 10^{-8} M leu-enkephalin, 10^{-7} M naloxone. Mean ± S.E.M. of 4-8 determinations.

*significantly different from A, C at $p<0.005$.

values (1.44 ± 0.10 nmol/mg tissue). The effect of leucine enkephalin was reversed in the presence of 10^{-7} M naloxone, which restored stimulated $^{45}Ca^{++}$ uptake to 2.17 ± 0.15 nmol/mg tissue. Leucine enkephalin and naloxone alone had no effect upon non-stimulated Ca^{++} accumulation.

DISCUSSION

Several lines of evidence suggest that enkephalin opiate peptides may be involved in cardiovascular regulation on a peripheral level. Methionine-enkephalin has been shown to produce a naloxone-reversible drop in perfusion pressure in the perfused cat hindlimb preparation as a result of vasodilation (7). In dogs, reserpine-pretreated to deplete adrenal catecholamines, stimulation of the splanchnic nerve produces a naloxone-reversible hypotension as a result of adrenal enkephalin release (8). Additionally, we have shown that enkephalins significantly attenuate the positive chronotropic response of isolated, spontaneously beating rat atria to (-)-NE, an effect reversed by naloxone (2).

Further investigation of this effect revealed that the attenuation of chronotropy by enkephalins in rat atria was specific for chronotropy induced by β-agonists. Chronotropic responses to the adenylate cyclase stimulating agent forscolin were unaffected by enkephalin (3). The attenuation of chronotropy was also found to be dependent upon external Ca^{++} concentration (3). The data suggested that

enkephalins might be attenuating NE-induced chronotropy by interferring with NE-stimulated Ca^{++} accumulation. Restriction of Ca^{++} flux had also been suggested as a mode of action of enkephalins in reducing ACh release at frog neuromuscular junction (4) and in reducing stimulated release of [^{3}H]-NE from rat cortical slices (5). Thus the present study was prompted.

The inhibition of NE-stimulated $^{45}Ca^{++}$ flux is quite pronounced with 10^{-8} M leu-enkephalin. We have previously reported (2) that the dose-effect curve for enkephalin-mediated attenuation of chronotropy in rat heart is biphasic. In that initial report, the maximal effect of leu-enkephalin was found to occur at 10^{-7} M. In the present study, the effect of leu-enkephalin was maximal at 10^{-8} M, both in attenuation of Ca^{++} flux and chronotropy (3). Other studies in this laboratory have required concentrations as high as 10^{-6} M. The reason for this variance is unclear. It is thus essential to examine a concentration range of enkephalins in such experiments.

The naloxone-reversible modulation of NE-stimulated Ca^{++} flux in rat atria further suggests the existence of specific interaction of enkephalins with adrenergic receptor-mediated events in rat heart. Although the nature of the interaction in unclear, a possible explanation can be suggested.

Adrenergic agonists promote calcium flux in cardiovascular tissue by activating a receptor-mediated Ca^{++} channel, and by releasing an agonist-releaseable Ca^{++} pool (see ref. 9 and ref. cited). The transduction of receptor occupancy to Ca^{++} flux and positive chronotropy in the heart is apparently adenylate cyclase-mediated (10). The data are consistent with a possible modulation by enkephalins of the coupling of the adrenergic receptor with the adenylate cyclase catalytic subunit. This possibility is consistent with our observation that chronotropy induced by forscolin, a direct stimulation of adenylate cyclase (11), is unaffected by enkephalins. Further experimentation will be required to confirm such a possibility.

These data, however, demonstrate an immediate effect of enkephalins on Ca^{++} metabolism in cardiovascular tissue which may be of both pharmacological and therapeutic interest.

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