EFFECT OF ENKEPHALIN AND SUBSTANCE P ON SYMPATHETIC NERVE TRANSMISSION IN MOUSE VAS DEFERENS

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Abstract—Effect of methionine-, leucine-enkephalin (met-, leu-enkephalin) and substance P on the transmission in mouse vas deferens was studied. Both met- and leu-enkephalin inhibited electrically induced contraction of vas deferens at \(10^{-8}\)-\(10^{-7}\) M, met-enkephalin being 1.4 times more active than leu-enkephalin. Nalorphine \(10^{-6}\) M antagonized these effects. Substance P \(10^{-9}\)-\(10^{-7}\) M had no effect on the contraction. Met- and leu-enkephalin \(10^{-7}\)-\(10^{-5}\) M decreased the high potassium induced \([\text{H}]\)-nor-epinephrine release from vas deferens, while substance P \(10^{-5}\) M significantly increased it. Nalorphine \(10^{-5}\) M reversed the inhibitory effect of met-enkephalin. These results indicate that these peptides modify the transmission of sympathetic nerve in mouse vas deferens.

A variety of evidence now indicates that a certain endogenous polypeptide in the central nervous system is involved in the regulation of sensory nerve transmission. Two peptides, methionine- and leucine-enkephalin (met- and leu-enkephalin) which have been suggested to be endogenous ligands for opiate receptors (1), are localized in synaptosomal fractions (2) and are thought to modulate the activity of the ascending pain pathway in the spinal cord and the brain. Substance P, undecapeptide isolated from bovine hypothalamus (3), is also localized in synaptosomal fractions (4, 5). Since substance P exerts a potent depolarizing action on the motoneurons and is localized for the greater part in the dorsal area of the dorsal horn, the role of an excitatory transmitter of primary afferent neurons was suggested (6).

Interestingly, these endogenous polypeptides modulate a stimulus-induced contraction of mouse vas deferens. Thus, both enkephalins depress the contraction (1) while substance P enhances it (7, 8). In all probability, the depression and the enhancement in contraction reflect a decrease and an increase of transmitter release from sympathetic nerves in the preparation, respectively. Henderson et al. (9) and Hughes et al. (10) demonstrated that morphine inhibits the transmitter release from mouse vas deferens. Henderson and North (11) and Henderson (12) indirectly suggested that normorphine and met-enkephalin depress the transmitter release from the vas deferens.

In planning the present experiments we attempted to confirm the findings that these peptides modulate the contraction of vas deferens and second, to demonstrate the influence
of these peptides on the potassium induced tritium release from the preparation previously loaded with \(^{3}\text{H}\)-norepinephrine (\(^{3}\text{H}\)-NE).

MATERIALS AND METHODS

L-NE-7-\(^{3}\text{H}\) (9.8 mCi/mmol) hydrochloride was obtained from the Radiochemical Centre, Amersham. Hexamethonium bromide was purchased from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Substance P, met- and leu-enkephalin were synthesized by the conventional method (13, 14). Nalorphine hydrochloride was generously donated by Prof. H. Takagi, of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University.

Male albino mice, weighing 15-25 g were decapitated and a pair of vasa deferentia was dissected out and mounted in an organ bath of 5 ml with modified Krebs solution (composition in mM per liter: NaCl 118; KCl 4.75; CaCl\(_2\) 2.54; KH\(_2\)PO\(_4\) 1.2; NaHCO\(_3\) 25; glucose 10) maintained at 37°C and aerated with 5% CO\(_2\) in O\(_2\). The intramural nerves were excited by rectangular pulses (50 msec, 0.1 Hz) of supramaximal strength applied between platinum ring electrodes placed vertically on opposite sides of the preparation. Contractions were recorded with an isometric transducer coupled to a Nihon Kohden Polygraph. To exclude the possibility that ganglionic synapses might play a role in the action of peptides, the experiments were performed in the presence of hexamethonium (28 \(\mu\)M). The tissue was set up and stimulated for 20 min and when the size of contraction had become constant, the substances to be tested were added and contraction was recorded for 2 min. Thereafter, the bath fluid was exchanged 3 times and the next substance for testing was added after 15 min.

For the measurement of \(^{3}\text{H}\)-NE from vas deferens, the tissues were incubated at 37°C for 30 min in modified Krebs solution containing hexamethonium (28 \(\mu\)M), ascorbic acid (0.1 mM), EDTA (0.03 mM) and \(^{3}\text{H}\)-NE (0.1 \(\mu\)M). At the end of labelling period, the preparations were washed 6 times for 60 min, after which new medium containing poly-peptides was introduced, the preparations were then incubated for 30 min with the fluid exchanged every 10 min for 20 min and every 5 min for a further 10 min. Thereafter, the tissues were incubated in the medium containing 40 mM KCl (40 mM NaCl was omitted from the medium) for 5 min. In each experiment, both media (0.5 ml) for 5 min before and after high potassium stimulation were collected in a scintillation vial containing 10 ml Bray's solution and the radioactivity was determined in a model 3320 Packard Tri-Carb liquid scintillation spectrometer and corrected for efficiency by external standardization. Counting efficiency was approximately 20%.

RESULTS

Effect of enkephalin and substance P on the electrically induced contraction of mouse vas deferens

Both met- and leu-enkephalin inhibited the contraction of the mouse vas deferens at an extremely low concentration (Fig. 1). The action was rapid in onset and lasted for more
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than 2 min. As is shown in Fig. 2, these inhibitory effects were dose-dependent (10^{-6}-10^{-7} M) and met-enkephalin was 1.4 times more active than leu-enkephalin. Nalorphine (10^{-6} M) had no effect on the contraction per se but antagonized considerably the inhibitory effects of met- and leu-enkephalin (Fig. 3). Substance P, at 10^{-9}-10^{-7} M had no effect on the contraction.

Fig. 1. The effect of met- and leu-enkephalin on the electrically induced contractions of mouse vas deferens. The intramural nerves were excited by rectangular pulses (50 msec, 0.1 Hz) of supramaximal strength.

Fig. 2. The effect of met- and leu-enkephalin on the electrically induced contractions of mouse vas deferens and the antagonistic effect of nalorphine. The stimulus conditions are the same as those in Fig. 1. Nalorphine was added 2 min before enkephalins.
Effect of enkephalin and substance P on the high potassium induced release of $[^{3}H]$-NE from mouse vas deferens

During 5 min incubation in normal modified Krebs solution, a spontaneous tritium release from a pair of vasa, which previously had accumulated $[^{3}H]$-NE, was $358.0 \pm 14.6$ cpm (mean ± S.E. of 15 experiments). In contrast, when the tissue was incubated with 40 mM KCl for 5 min the release increased to $1150.5 \pm 80.1$ cpm. The difference between these two releases, $792.5 \pm 76.6$ cpm, is hereafter referred to as potassium induced release. Effects of peptides on the potassium induced release are summarized in Table 1. Progressive decrease in the release occurred with increasing concentration of met-enkephalin ($10^{-7}$-$10^{-5}$ M). Leu-enkephalin ($10^{-5}$ M) inhibited the release by about 50%, the effect being equipotent with that of met-enkephalin. Substance P ($10^{-5}$ M) significantly increased the release. Nalorphine ($10^{-5}$ M) by itself had no effect on the release but when nalorphine ($10^{-5}$ M)
and met-enkephalin (10^-8 M) were added simultaneously to the medium, the inhibitory effect of met-enkephalin was reversed incompletely but significantly (Table 1). None of these substances affected a spontaneous tritium release at the concentration which induced certain changes in the potassium induced release.

**DISCUSSION**

Our experiments show that met- and leu-enkephalin inhibited the contraction of the mouse vas deferens elicited by field stimulation. The ID50, calculated from the dose-response curve, was 4.3×10^-8 M for met-enkephalin and 6.0×10^-8 M for leu-enkephalin (Fig. 2), the results being similar to those reported by Hughes et al. (1). On the other hand, the result that substance P had no effect on the contraction at 10^-7 M differs from observations made by Euler and Hedqvist (8) who found that substance P (approximately 10^-10 M) enhanced the stimulus response in the guinea pig vas deferens. Experimental conditions and animal species no doubt contributed to the discrepancy.

Preliminary studies (unpublished) showed that electrical stimulus which could elicit a considerable contraction of mouse vas deferens, failed to release a detectable amount of [3H]-NE. Therefore, potassium depolarization was applied when the effect of the peptides on tritium release was examined. There is direct evidence suggesting that potassium depolarization is more specific for transmitter release than electrical stimulation, *in vitro*. Bennet et al. (15) observed that glycine, a putative transmitter in the spinal cord, could be released from the spinal cord but not from the cerebral cortex with a high potassium preparation. In contrast, electrical stimulation of the cerebral cortex or spinal cord induced release of almost all amino acids. Furthermore, Thoa et al. (16) found that depolarization of the vas deferens *in vitro* by high potassium produced a NE release from sympathetic nerve terminals by exocytosis.

Both met- and leu-enkephalin reduced the potassium induced tritium release without affecting the spontaneous release. Hughes et al. (10) demonstrated that morphine depressed the electrically evoked outflow of catecholamine from mouse vas deferens but was completely without effect on the resting outflow. A similar conclusion was reached by Henderson and North (11) and Henderson (12) who studied the effect of normorphine and met-enkephalin on excitatory junction potential in mouse vas deferens. Therefore, our results are essentially similar to their findings.

The effective concentrations which could reduce the potassium induced release were somewhat higher than those required for the inhibition of contraction. This may imply that undetectable small changes in the amount of NE released from sympathetic nerve terminals contribute to the changes in muscle contraction. The inhibitory effects of met-enkephalin on both muscle contraction and transmitter release were antagonized by nalorphine, hence it is probable that these effects are mediated by opiate receptors. Taube et al. (17) have recently shown that in rat brain slices, met-enkephalin diminished the overflow of [3H]-NE evoked by electrical stimulation or by high potassium and the effect was antagonized by naloxone. Our results are essentially similar to these observations.
Euler and Hedqvist (8) suggested that substance P enhances the stimulus response in the vas deferens by partial depolarization of the muscle membrane. Our present experiments in the mouse vas deferens clearly demonstrated that substance P increased high potassium induced NE release which was probably caused by exocytosis. Precise site and mode of this action remain to be determined but the findings imply that substance P stimulates sympathetic nerves in the vas deferens. Such would also be in accordance with the observations by Magnusson et al. (18) who found that substance P has a stimulatory action on monoaminergic neurons in the brain.

In summary, met- and leu-enkephalin decreased the potassium induced release of NE from mouse vas deferens while substance P increased it. This is the first demonstration of a modulation of NE release from vas deferens by endogenous polypeptides which are involved in the regulation of sensory nerve transmission.

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