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Rapid communication

POTENTIATION BY NALOXONE OF REFLEX ACTIVITY IN THE ISOLATED SPINAL CORD OF *XENOPUS*

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Opiate receptors, and enkephalins which are endogenous ligands for these receptors, are found in many parts of the vertebrate nervous system (Snyder and Simantov, 1977). A high receptor density together with a high concentration of enkephalins occurs in the dorsal horn of the spinal cord and is probably associated with primary afferent nerve terminals and interneurons (Atweh and Kuhar, 1977; Hökfelt et al., 1977). The widespread distribution of the enkephalins makes it highly unlikely that their only physiological function is in the control of pain perception. Recently it has been suggested that the enkephalins act as inhibitory neurotransmitters (Frederickson, 1977). If the enkephalins have a more general physiological function, the application of pure narcotic antagonists such as naloxone should procedure significant effects. So far, however, evidence for such direct actions by narcotic antagonists is rather scarce (Frederickson, 1977).

We report here that naloxone in concentrations starting as low as 5×10^{-8} M potentiated the reflex activity in the isolated spinal cord of *Xenopus*. This effect was concentration-dependent and could be reversed by D-ala-met-enkephalinamide.

The experiments were carried out on the isolated spinal cord of the clawed frog, *Xenopus laevis*, at a temperature of 20 ± 1°C. The caudal part of the spinal cord with spinal roots IX and X was dissected by dorsal laminectomy from a spinal animal anaesthetized with tricaine methanesulfonate (MS 222, Sandoz). The spinal cord was placed on its side in a central groove of an experimental chamber, which was continuously perfused with standard frog Ringer solution. The spinal roots were pulled through silicone-sealed slits on either side of the groove and mounted on a pair of silver wire electrodes. Either the IXth or Xth dorsal root was stimulated at 1 min^{-1} with supramaximum pulses of 0.1 msec duration. Ventral root responses were recorded from the ipsilateral ventral root by a differential, ac-coupled amplifier (time constant 0.1 sec), displayed on an oscilloscope and digitized. Five successive responses were averaged by computer and plotted. Naloxone hydrochloride (ACF) and D-ala-met-enkephalinamide (Beckman) were applied to the spinal cord by switching from normal superfusion to Ringer solution which contained the desired concentration of the drug.

A single stimulus to the dorsal root evoked a synchronized monosynaptic response in the ventral root, followed by polysynaptic activity. Shortly after the application of naloxone, the amplitude of the ventral root response started to increase. The effect of different concentrations of naloxone on the peak amplitude of the monosynaptic response is illustrated in fig. 1. A concentration of 5×10^{-8} M produced a slight increase in the monosynaptic response, which did not differ significantly from the control experiments. Naloxone concentrations between 8×10^{-8} M and $5 \times$

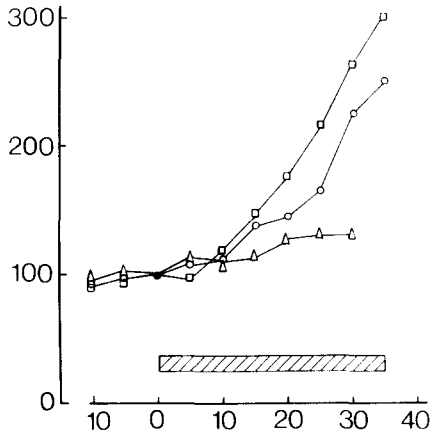

Fig. 1. Potentiation of the monosynaptic ventral root response of the isolated spinal cord of *Xenopus* by various concentrations of naloxone. Naloxone was applied at time 0 for a period of 35 min as indicated by the hatched bar. Amplitudes of monosynaptic responses are expressed as a percentage of the amplitude at time 0. (△) 5×10^{-8} M, n = 2; (○) 8×10^{-8} M—5×10^{-7} M, n = 5 and (□) 10^{-6} M naloxone, n = 3. *Ordinate*: amplitude (%); *abscissa*: time (min).

10^{-7} M caused a marked potentiation of the monosynaptic response and after 10^{-6} M naloxone, its amplitude increased to 300% of the value prior to the application of naloxone.

The effect of naloxone could not be reversed by washing with normal Ringer solution. Although the increase in amplitude leveled off, the monosynaptic response did not start to decrease, indicating that naloxone was not easily washed out. Therefore, an attempt was made to reverse the effect of naloxone by treating the preparation with D-ala-met-enkephalinamide, which on its own produces a potent suppression of spinal reflex activity that is rapidly reversed by washing with normal Ringer (W. Wouters et al., in preparation). After application of D-ala-met-enkephalinamide at 10^{-5} M for 10 min, followed by a 50 min washing period with normal Ringer, the amplitude of the monosynaptic response decreased to the control level from a value of 274 ± 15% (mean ± s.d.; n = 3) just before the application of D-ala-met-enkephalinamide. The complete reversal of the naloxone-induced potentiation by a short treatment with D-ala-met-enkephalinamide strongly suggests that the effect of naloxone is mediated via specific opiate receptors. Naloxone may potentiate the spinal reflex activity by antagonizing the inhibitory action of endogenous enkephalins or other endorphins which may be present in high levels because of the stress induced by the dissection. A dose-independent increase in spinal reflex activity has been observed in the acute spinal cat after i.v. injection of naloxone and may be due to a similar mechanism (Goldfarb and Hu, 1976). The present results clearly demonstrate that naloxone alone produces a significant effect in an isolated preparation and that this effect can be reversed by D-ala-met-enkephalinamide.

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