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BLOCK OF THE HYOSCINE-RESISTANT OPIATE WITHDRAWAL CONTRACTURE OF ILEUM BY A NEW SUBSTANCE P ANTAGONIST [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]SUBSTANCE P

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The isolated guinea-pig ileum is a classic preparation for studying opiate dependence (Collier et al., 1981). The opiate antagonist naloxone elicits in the tolerant-dependent ileum a powerful contracture which consists of a cholinergic and a non-cholinergic (hyoscine-resistant) component. Previous studies attributed this non-cholinergic contracture to substance P release since it was blocked by substance P tachyphylaxis (Gintzler, 1980; Tsou et al., 1982). With the advent of substance P antagonists, it became of interest to test the hypothesis by using such a tool. Therefore, the new substance P antatonist [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P was used in the present work to see if it could block the hyoscine-resistant naloxone-precipitated withdrawal contracture.

Guinea-pigs of either sex (250-300 g) were implanted subcutaneously with one morphine pellet containing 75 mg morphine hydrochloride. The animal was killed by a blow to the head on the third day after overnight fasting. The ileum was removed, the terminal 10 cm of the ileum was discarded and the rest kept in oxygenated Krebs-Ringer buffer containing 0.1 μM morphine hydrochloride to prevent withdrawal effects.

Three adjacent strips of the longitudinal muscle myenteric plexus (2.5 cm each) were prepared and mounted in parallel in three double-jacketed 8 ml baths maintained with Krebs-Ringer at 37°C and gassed with 95% O_2-5% CO_2. The muscle strips were connected to isotonic force transducers (TD-112S) coupled to a Nihon Kohden Polygraph. After mounting, the preparations were allowed to equilibrate in the buffer for 1 h with fresh changes every 15 min. Electric field stimulation (0.1 Hz, 1 ms, supramaximal voltage) was delivered via circular electrodes.

The drugs used were: morphine hydrochloride (Qinhai Pharmaceutical Factory), hyoscine bromide (BDH), substance P (Sigma); [D-Arg^1,D-Phe^5,D-Trp^{7,9},Leu^{11}]substance P which was synthesized by one of us (Y.-A. Lu, unpublished, 1983), and was found to be a highly potent competitive substance P antagonist in the guinea-pig ileum (pA_2 8.14, Tan et al., unpublished).

Fig. 1 shows a typical experiment. After twitches in response to electric stimulation had been obtained for about 30 min, the stimulation was terminated and hyoscine was added in the first two baths (A and B) to make a final concentration of 2 μM. This concentration sufficed to block completely the spasmogenic activity of 1 μg acetylcholine. Five minutes later, the substance P antagonist was added to the second (B) and third (C) baths to make a final concentration of 0.02 mM. After another 5 min, naloxone was added to all three baths to make a final concentration of 0.1 μM.

The non-cholinergic naloxone-precipitated withdrawal contracture gradually attained its peak in 1 min as shown in fig. 1A. Fig. 1C shows that when the preparation was pretreated with the substance P antagonist, the contracture was much higher in amplitude and attained its peak almost instantly. The duration of the contracture, however, was about the same. When the preparation was pretreated with both hyoscine and the substance P antagonist, the withdrawal contracture

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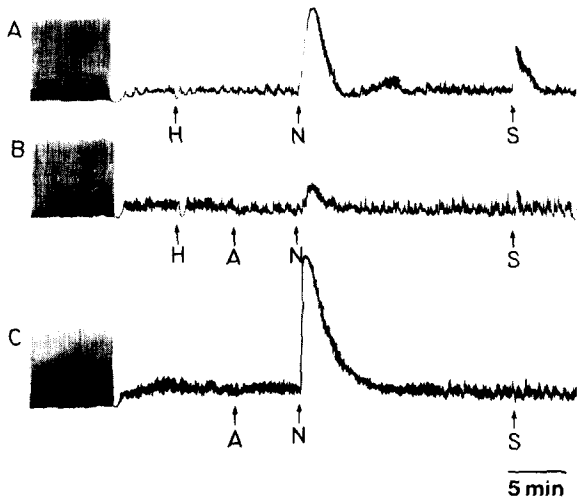

Fig. 1. Blockade of hyoscine-resistant opiate withdrawal contracture of guinea-pig ileum by substance P antagonist. (A) Naloxone-precipitated withdrawal contracture after hyoscine. This preparation remained responsive to substance P. (B) The naloxone-precipitated withdrawal contracture became very small after hyoscine and substance P antagonist [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]substance P. (C) Naloxone elicited a contracture after substance P antagonist alone. Preparations B and C did not respond to substance P. H: hyoscine 2 μM; A: substance P antagonist 0.02 mM; N: naloxone 0.1 μM; SP: substance P 1.5 nM.

became very small (fig. 1B) or even vanished. After the naloxone-precipitated contracture had waned, substance P was added to all three baths to make a final concentration of 1.5 nM. Only the muscle strip in the first bath responded with a contraction, indicating the effectiveness of the substance P antagonist on the guinea-pig ileum.

The above results confirmed that the hyoscine-resistant (non-cholinergic) gut opiate withdrawal contracture was largely due to substance P release.

References

Collier, H.O.J., N.J. Cuthbert and D.L. Francis, 1981, Model of opiate dependence in the guinea-pig isolated ileum, Br. J. Pharmacol. 73, 921.

Gintzler, A.R., 1980, Serotonin-substance P pathway in the manifestation of gut withdrawal, in: Endogenous and Exogenous Opiate Agonist and Antagonists, ed. E. Leong Way, Pergamon Press, New York, p. 497.

Tsou, K., G. Louie and E.L. Way, 1982, Manifestations of gut opiate withdrawal contracture and its blockade by capsaicin, European J. Pharmacol. 81, 377.