Short communications

Effects of a morphine-rabbit anti-morphine antibody mixture on guinea-pig isolated ileum

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Incubates of morphine with serum globulins obtained from sera of rabbits immunized with a morphine-bovine serum albumin conjugate produced immediate Schultz-Dale contractions when added to superfused, electrically stimulated guinea-pig ileal strips. Incubates of morphine with Krebs-Henseleit solution produced relaxation and depression of tone, and inhibition of electrically induced contractions. It is concluded that the spasm of guinea-pig ileum produced by incubates containing morphine-binding serum globulins and morphine resulted from transient passive sensitization and an acute anaphylactic type of response.

Several recent studies have established that morphine will function antigenically when conjugated to an appropriate carrier molecule (Spector & Parker, 1970; Van Vunakis, Wasserman & Levine, 1972; Wainer, Fitch, Rothberg & Schuster, 1973). An antibody-like, morphine-binding immunoglobulin has been detected after repeated daily injections of rabbits with morphine sulphate or morphine hydrochloride (Ringle & Herndon, 1972) and significant binding of morphine by serum globulins of heroin addicts has been described (Ryan, Parker & Williams, 1972). Using an *in vitro* system, we found that exposure of normal guinea-pig ileum to incubation mixtures containing morphine and anti-morphine rabbit serum globulins provokes typical Schultz-Dale contractions *in vitro* and also inhibited the suppressive action of morphine on electrically-induced muscle contractions.

Methods.—Anti-morphine sera were obtained from rabbits immunized with a morphine-bovine serum albumin conjugate which was prepared by methods described previously (Spector & Parker, 1970). All sera were analysed for anti-morphine reactivity by the use of [^{14}C]-*N*-methyl morphine in a modified primary binding test (Brandt, Wyle & Artenstein, 1972). One ml of undiluted, pooled antiserum was found to bind approximately 2·8 μg of morphine. The pooled antiserum and a pool of normal sera were subjected to ammonium sulphate fractionation to remove serum albumin. The globulins were precipitated and washed once with 50% ammonium sulphate, dissolved in 0·9% w/v NaCl solution (saline), dialysed overnight at 5° C against deionized water and freeze-dried. Before testing, the globulins were reconstituted in saline to the original volume. One ml of the undiluted pooled antibody globulins was found to bind approximately 2·7 μg of morphine while the pool of normal rabbit serum globulins had no significant morphine binding capacity.

The responses of guinea-pig ileum were tested by a superfusion technique which was a modification of that described by Gaddum (1953). Guinea-pigs were killed by a blow on the back of the head. A strip of terminal ileum, beginning 10 cm from the ileo-caecal junction and approximately 15 cm long, was rapidly removed, suspended in air and moistened by a continuous drip (approximately 5 ml/min) of oxygenated Krebs-Henseleit solution warmed to 37° C. The strip was stimulated by electrical pulses applied along the length of the tissue. Pulses were provided by the stimulator portion of a Heath Impscope using an external capacitor of 1·0 ufd for a time constant which yielded 18·5 pulses/min of 0·2 s duration. When set at maximum output of 50 V, the stimulator developed non-ideal square wave pulses with a very rapid rise time. A weighted isotonic lever system (4·5 to 1 ratio) was linked to a transistorized displacement transducer to detect contractile activity which was monitored on a Brush Mark 260 recorder.

A single ileal strip was usually employed for a series of determinations. After a baseline of activity, induced by electrically-induced contractions, was obtained, an aliquot of a test mixture was pipetted directly onto the tissue. The test material was subsequently removed by the con-

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tinuous flow of Krebs-Henseleit solution, and the tissue was allowed to stabilize to the baseline tone and contractile activity before another test mixture was added.

Test mixtures were prepared in silicone-coated tubes; varying volumes of a stock solution (10 μg/ml) of morphine sulphate were added to individual tubes to provide a morphine concentration range of 100 to 1,000 ng/tube. Test sera were added in 0·1 ml aliquots to each tube and Krebs-Henseleit solution was added to produce a final volume of 0·2 ml. The mixtures were incubated at room teperature for 3 h after which they were diluted to 1 ml with Krebs-Henseleit solution and assayed in 0·1 ml aliquots immediately or after storage at −20° C.

Results.—Representative recordings of ileal contractions obtained in a typical experiment are shown in Figure 1. To produce these tracings, 0·1 ml of either immune or normal rabbit serum globulins were incubated with 0·1 ml (1 μg) of morphine and diluted to 1 ml before assay, and a 0·1 ml aliquot added to the strip. Morphine incubated with diluent served as a control. It was consistently noted that addition of the mixtures containing morphine and the globulin fraction of the anti-morphine serum pool (IRSG) induced an immediate contraction of the muscle and, after recovery, the electrically-induced contractions resumed (Figure 1a). In contrast, a loss of muscle tone occurred and neither the immediate contractions nor the electrically-induced contractions were observed with a mixture of morphine and normal globulins (NRSG) or with morphine alone, as shown, respectively, in Figure 1b and 1c. The immediate contractions were also not observed on separate additions of the globulin fractions followed by additions of morphine sulphate.

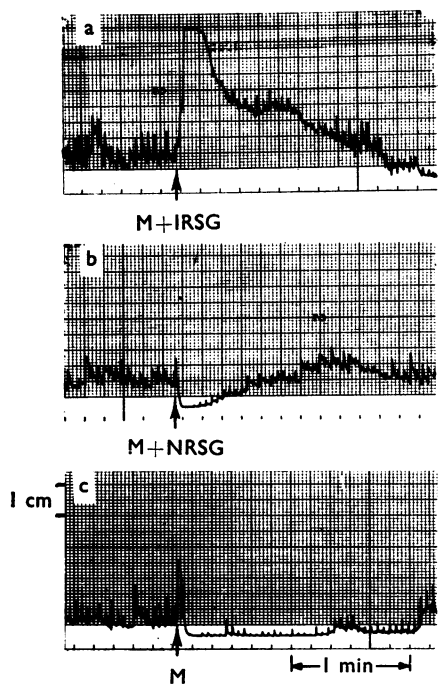

FIG. 1. Patterns of electrically-induced contractions of normal guinea-pig ileum influenced by mixtures of morphine (M) and rabbit anti-morphine serum globulins (IRSG)—(a), morphine (M) and normal rabbit serum globulins (NRSG)—(b), and morphine (M) alone—(c).

Discussion. — It seems reasonable to attribute the responses to the IRSG mixtures to the presence of a morphine-antibody complex and to consider the early spasm as representing a Schultz-Dale reaction which resulted from immediate and transient passive sensitization of the tissue

with this complex. Trapani, Garvey & Campbell (1958) described similar spasms of smooth muscle of guinea-pig ileum following additions of soluble antigen-antibody complexes formed in the presence of excess antigen. Trapani *et al.* (1958) tested the effects of solutions of bovine serum albumin (BSA) and rabbit anti-BSA antiserum. The present experiments differ since the smooth muscle spasm resulted from addition of a haptene-antibody mixture, not the original sensitizing antigen. The failure to detect a similar response on separate exposures of the muscle to anti-morphine globulins followed by morphine might be explained by the quick removal of these reactants by the continuous washing of the tissue with Krebs-Henseleit solution. The response to the morphine antibody mixture appears to reflect the direct binding of morphine to the antibody globulins to form soluble complexes.

Although electrical pulses of long duration were used which may have stimulated the muscle directly, morphine has previously been shown to be inhibitory to direct acting smooth stimulants as well as to indirect stimulation (Lewis, 1960). The failure of Wainer *et al.* (1973) to detect the Schultz-Dale contractions with an incubation mixture containing morphine and rabbit anti-morphine globulins might be attributed to differences in the design of their experiments and apparatus. The electrical impulses which they applied to the muscle strip coaxially produced rapid and maximal contractions and were detected by a pressure transducer. Under these conditions, the relatively slow and sustained early contraction which we observed may well have been obscured. The procedure followed by Wainer *et al.* (1973) included the use of an antihistamine in the bath.

The relationship of these observations to the effects of morphine *in vivo* is not known. The present observations may merely represent another means of demonstrating a specific antigen-antibody reaction and have no pharmacological significance in the intact animal. However, unexplained acute heroin deaths have been described, and these observations suggest that acute anaphylactic reactions may be involved (A. Goldstein, personal communication) since there is significant binding of morphine to the γ-globulin fraction of addict sera to form a non-dialysable complex, which does not occur in normal controls (Ryan *et al.*, 1972).

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REFERENCES

BRANDT, B. L., WYLE, F. A. & ARTENSTEIN, M. S. (1972). A radioactive antigen-binding assay for *Neisseria meningitidis* polysaccharide antibody. *J. Immunol.* **108**, 913–920.

GADDUM, J. H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.* **8**, 321–326.

LEWIS, G. P. (1960). The inhibition by morphine of the action of smooth muscle stimulants on the guinea-pig intestine. *Br. J. Pharmac. Chemother.* **15**, 425–431.

RINGLE, D. A. & HERNDON, B. L. (1972). *In vitro* morphine binding by sera from morphine-treated rabbits. *J. Immunol.* **109**, 174–175.

RYAN, J. J., PARKER, C. W. & WILLIAMS, R. C. (1972). γ-Globulin binding of morphine in heroin addicts. *J. Lab. Clin. Chem.*, **80**, 155–164.

SPECTOR, S. & PARKER, C. W. (1970). Morphine: radioimmunoassay, *Science*, **168**, 1347–1348.

TRAPANI, I. L., GARVEY, J. S. & CAMPBELL, D. H. (1958). Stimulating action of soluble antigen-antibody complexes on normal guinea-pig smooth muscle. *Science*, **127**, 700–701.

VAN VUNAKIS, H., WASSERMAN, E. & LEVINE, L. (1972). Specificities of antibodies to morphine. *J. Pharmac. exp. Ther.*, **180**, 514–521.

WAINER, B. H., FITCH, F. W., ROTHBERG, R. M. & SCHUSTER, C. R. (1973). *In vitro* morphine antagonism by antibodies. *Nature*, **241**, 537–538.

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