produced a significant increase in quinidine toxicity but there was a barely significant interaction with xylocaine.

In conclusion, a marked synergism has been shown to occur between propranolol and various anaesthetics and morphine. This synergism was not seen with D(−)-INPEA. Unlike propranolol, D(−)-INPEA does not have depressant actions on the central nervous system nor quinidine-like actions on the heart—effects of propranolol which may be involved in its synergism with the anaesthetics and morphine.

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The effects of morphine, pethidine and nalorphine on the isolated frog skin preparation

SIR,—Although morphine has a long history of clinical use, the mechanism by which it exerts its important and complex effects upon the central nervous system is still obscure. As an alternative to direct studies on the neuraxis, experimentally simpler systems shown to be affected by morphine have been examined, e.g. guinea-pig ileum (Paton, 1957; Cox & Weinstock, 1966) and superior cervical ganglion (Kosterlitz & Wallis, 1966). The present work arose from the chance observation that morphine produced effects upon the frog skin preparation. The actions of some nitrogenous bases on the transport of sodium ions across this membrane have been studied by Kirschner (1953) and Skou (1961). In the following experiments, morphine, pethidine and nalorphine were applied to the isolated skin.

A circle of washed abdominal skin of *Rana temporaria* separated frog Ringer solution (pH 7·65) contained in two adjacent 15 ml cells at room temperature. The preparation was left for 2 hr to equilibrate and then the short-circuit current (SCC) which had to be applied to reduce the skin potential to zero was measured. The current was maintained continuously for the rest of the experimental period, adjustments and readings being made at 5 min intervals. Drugs were applied to either surface of the membrane and any changes in the SCC noted.

Fig. 1 shows typical results following application to the inside of the skin. The three drugs produced significant falls in SCC, approximately equipotent doses being morphine sulphate 10 mg, pethidine hydrochloride 1 mg and nalorphine hydrobromide 5 mg (corresponding to the following final concentrations in the bathing solution in terms of the free bases: morphine 1·75 mM, pethidine 0·23 mM and nalorphine 0·85 mM). Although different preparations varied in sensitivity, the initial value of the SCC did not appear to be critical provided that it was greater than 80 μA/4 cm^2. When the same drugs were applied to the outer surface of the frog skin, larger doses (4–10 times) were required to produce significant falls in the SCC. It will be seen from Fig. 1

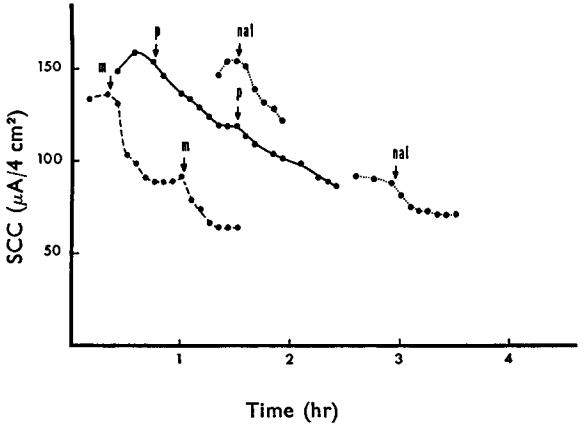

FIG. 1. The effects on the short-circuit current (scc) across the isolated frog skin of two separate doses of morphine (m, 10 mg, dashed line), pethidine (p, 1 mg, unbroken line) or nalorphine (nal, 5 mg, dotted lines) applied to the inner surface.

that a second dose of morphine or pethidine given 40–45 min after the original dose of the same drug produced a similar quantitative effect. The responses to 10 mg morphine or 1 mg pethidine were not markedly modified by a single dose of 5 mg nalorphine given either simultaneously or in the preceding or the following 30 min. The concentrations of morphine necessary to produce the above effects are much larger than those required to depress transmission at post-ganglionic neuro-effector junctions (Cox & Weinstock, 1966) or at autonomic ganglia (Kosterlitz & Wallis, 1966) but of a similar order to those found to be effective for the actions of amines on the isolated frog skin preparation (Skou, 1961).

Skou (1961) found that the action of amines applied to the outside of the frog skin was pH dependent, i.e. it varied with the degree of ionic dissociation of the drug. As morphine and pethidine are weak bases with pK_a values above 7·65 (Beckett, 1956), in frog Ringer solution they are mainly present in the ionised form. The pK_a value and the % ionisation at pH 7·65 and 9·05 are respectively for pethidine, 8·72, 92, 32, and for morphine, 8·05, 72, 9. Thus to observe what happened when excess of the unionised base was present at the inner surface of the membrane, the experiments were repeated in frog Ringer solution buffered to pH 9·05 by the addition of 50 mM Tris[2-amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride] and 5 mM HCl. To avoid calcium precipitation at such an alkaline pH, the concentration of this ion was reduced to one-tenth of the usual: a control experiment showed that this alone did not affect the drug responses. At pH 9·05 the responses to 10 mg morphine and to 1 mg pethidine were almost identical with those obtained at pH 7·65. There is no evidence, therefore, that the ionised and unionised forms of morphine or pethidine have significantly different effects when applied to the inside of the frog skin preparation.

On the accepted model of the frog skin (Koefoed-Johnsen & Ussing, 1958), vasopressin is considered specifically to increase the permeability of the membrane selectively permeable to sodium at the outer surface of the basal epithelial cell ("sodium permselective membrane") and ouabain specifically to depress the

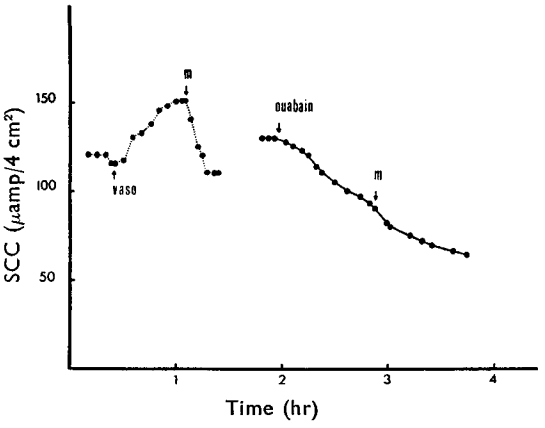

FIG. 2. The effects of morphine (m, 10 mg) on the short-circuit current (scc across the isolated frog skin following either vasopressin (vaso, 10 units, dotted line or ouabain (1 μg, unbroken line). All drugs applied to the inner surface of the membrane.

Na^+ K^+ Mg^{++}-dependent ATPase ("sodium pump") located in the inward-facing surface of the same cell. Therefore, in an attempt to define more precisely the action of morphine on this preparation, the drug was applied after either vasopressin (10 units) or ouabain (1 μg). Typical results are shown in Fig. 2, from which it will be seen that whereas after vasopressin the morphine effect was *practically unchanged*, after ouabain the response was insignificant. These findings suggest that morphine may be acting primarily on the sodium pump mechanism. However, the effect of morphine on the frog skin scc may well be a non-specific one in view of the large concentrations of drug necessary to produce it in comparison with the minimal effective doses at postganglionic neuro-effector junctions (Cox & Weinstock, 1966; Kosterlitz & Wallis, 1966).

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