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Effects of narcotic analgesics on contractions of the chick isolated, innervated oesophagus

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The effects of the analgesics, morphine, diacetylmorphine, etorphine, methadone, pethidine, propoxyphene, pentazocine, cyclazocine and phenazocine were studied on contractions of the isolated innervated oesophagus of chicks. All of the analgesics depressed contractions of the oesophagus evoked by coaxial stimulation, the effect being greater the lower the frequency of stimulation. Tachyphylaxis and cross tachyphylaxis to and between the drugs developed rapidly. During the depressant effect on the electrically evoked contractions, responses to acetylcholine and 5-hydroxytryptamine were not depressed, except in the cases of pethidine, propoxyphene and pentazocine which depressed responses to acetylcholine. Nalorphine added during the depressant action antagonized the effects of morphine, diacetylmorphine, etorphine, methadone and phenazocine, partially antagonized those of pethidine and propoxyphene but did not antagonize those of pentazocine or cyclazocine.

The actions of morphine and related analgesics have been extensively studied on the isolated ileum of the guinea-pig (Trendelenburg, 1917; Schaumann, Giovannini & Jochum, 1952; Schaumann, 1954, 1955, 1956a & b; 1957: Kosterlitz & Robinson, 1955; Paton, 1957, 1963, 1969; Kosterlitz & Taylor, 1959; Gyang & Kosterlitz, 1966; Cox & Weinstock, 1966; de la Lande & Porter, 1967; Paton & Zar, 1968; Fennessy, Heimans & Rand, 1969; Harris, Dewey & others, 1969). The analgesic drugs depress the response of the ileum to transmural stimulation, the effect being more marked the lower the frequency of stimulation. The response to acetylcholine is not depressed. Taken together, the results of the above-named authors show that the analgesic drugs impair part of the mechanism for acetylcholine release from intramural nerves, probably by an action on the axonal membranes. In the experiments now described, the effects of analgesic drugs were examined on a different cholinergically innervated smooth muscle preparation, the isolated upper oesophagus of the chick (Bowman & Everett, 1964; Everett, 1966, 1967). It was considered that this preparation might possess some advantages over the guinea-pig ileum, since it may be excited to contract both by stimulation of its extrinsic parasympathetic nerve supply and by transmural stimulation, and it does not possess an adrenergic innervation (Everett & Mann, 1967) which might otherwise complicate its responses.

METHODS

The method used was similar to that described by Bowman & Everett (1964). White leghorn chicks aged between 1 and 12 days were starved overnight and then killed with ether. The upper oesophagus as far as the crop was removed together with the right parasympathetic nerve trunk which runs along the course of the jugular vein. Apart from about a 1 cm length at the free end, the nerve was not separated from the jugular vein so that damage to the fine nerve branches passing to the oesopha-

gus was avoided. The preparation was suspended in Krebs solution (g/litre: NaCl, 6·95; KCl, 0·34; $CaCl_2$, 0·28; KH_2PO_4, 0·162; $MgSO_4$, 0·294; $NaHCO_3$, 2·1; dextrose 2·0) continuously gassed with 5% carbon dioxide in oxygen and maintained at 32°. The nerve was passed through stimulating electrodes of the type described by Burn & Rand (1960), and was stimulated with rectangular pulses of 0·5 ms duration and of a strength greater than that necessary to produce a maximal contraction at the frequency of stimulation used. Stimulation was also applied coaxially between an electrode (the anode) in the lumen and a second electrode in the external fluid. Contractions were recorded on smoked paper with an isotonic frontal writing lever loaded with 2g and amplifying the contractions 10 times.

The drugs used were morphine hydrochloride and diacetylmorphine hydrochloride (Macfarlane Smith), methadone hydrochloride, pethidine hydrochloride and nalorphine hydrobromide (Burroughs Wellcome), etorphine hydrochloride (Reckitt & Son), dextropropoxyphene hydrochloride (Eli Lilly), acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate and atropine sulphate (British Drug Houses), hexamethonium bromide (May & Baker), methysergide (Sandoz), phenoxybenzamine hydrochloride (Smith, Kline & French), mecamylamine hydrochloride (Merck, Sharp & Dohme). (±)-Phenazocine hydrobromide, (±)-pentazocine base, and (±)-cyclazocine base were supplied by Dr. R. T. Parfitt of the Department of Pharmaceutical Chemistry, University of Strathclyde. The concentrations given in the text refer to the bases or the cations.

RESULTS

The chick isolated oesophagus does not respond to a single nerve shock and therefore it was not possible to use stimulation frequencies as low as those employed with the guinea-pig ileum (0·1 Hz) by other workers. The lowest frequency of stimulation necessary to evoke reproducible responses of the oesophagus was 1 Hz and this was therefore the lowest frequency used.

Morphine depressed the responses of the oesophagus both to transmural and to extrinsic nerve stimulation. The depressant effect was more pronounced the lower the frequency of stimulation, within the range 1–10 Hz (Fig. 1a). Morphine is also

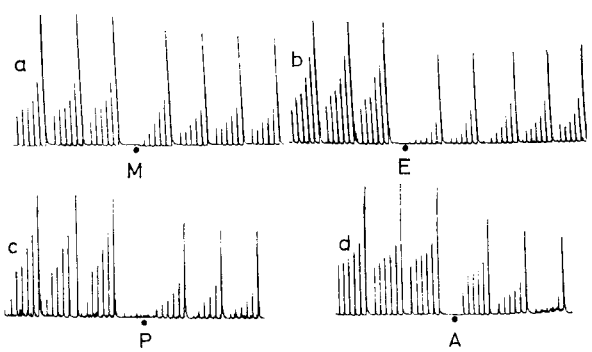

FIG. 1. a, b, c, and d are from different experiments. Each preparation was stimulated coaxially for 10 s every 60 s. The frequencies were 1, 2, 3, 4, 5 and 10 Hz. At M, 5 μg/ml morphine; at E, 5 μg/ml etorphine; at P, 5 μg/ml pethidine; and at A, 0·1 μg/ml atropine. The responses to the lower frequencies were always the more depressed. With the analgesics, the maximum effect was reached quickly, and with morphine and etorphine the lower frequency contractions then partially recovered. The effects of pethidine and atropine were maintained. The maximum effect of atropine was relatively slow in developing.

more effective in depressing the guinea-pig ileum at lower frequencies of stimulation (Paton, 1957).

In the guinea-pig ileum the depressant action is detectable with concentrations as low as 10 ng/ml and reaches a maximum at around 10 μg/ml (Paton, 1969). The chick oesophagus was much less senstitive, concentrations of morphine below 1 μg/ml being without depressant effect, although they occasionally produced a slight augmentation. Concentrations of 1 μg/ml and above depressed the contractions, the effect occasionally being preceded by slight augmentation of one or two responses. The depression was never complete even with the highest initial doses added (up to 100 μg/ml) and the lowest frequency of stimulation used (1 Hz). In most preparations the maximum depression did not exceed 50%, and in a few preparations out of 280 studied, especially in the winter months, morphine was without depressant effect on the responses to transmural or extrinsic nerve stimulation in all concentrations used (up to 100 μg/ml). There was a strong impression that morphine was more consistently effective in depressing the contractions during the summer months.

When responses to transmural stimulation were recorded alternately with responses to extrinsic nerve stimulation, it was consistently observed that morphine depressed the transmurally-evoked responses to the greater extent (Fig. 2a).

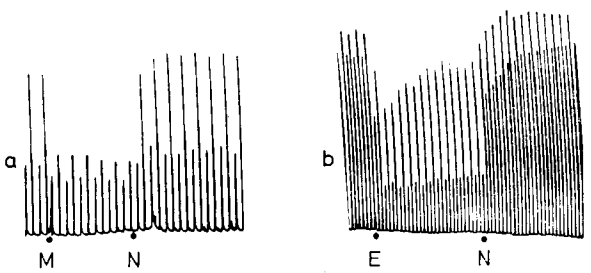

FIG. 2. a and b are from different experiments. The preparations were stimulated alternately via the extrinsic nerve and coaxially (2 Hz for 10 s every 60 s). In a, the bigger contractions are to coaxial stimulation; in b, the smaller contractions are to coaxial stimulation. At M, 5 μg/ml morphine; at E, 5 μg/ml etorphine; at N, 5 μg/ml nalorphine. Both morphine and etorphine were more effective in depressing responses to coaxial stimulation.

When morphine was left in contact with the tissue, an initial rapid but partial recovery towards the control amplitude usually occurred especially at low frequencies of stimulation (Fig. 1a). Rate of recovery then slowed and in many cases, especially with transmural stimulation, complete recovery was never achieved even after repeated and prolonged washing. Recovery of responses to extrinsic nerve stimulation was usually more complete than that of responses to transmural stimulation.

The depressant effect of atropine resembled that of morphine in that it was more pronounced against low frequencies of stimulation. However, it differed from that of morphine in that it was slower in onset and no partial recovery or tachyphylaxis was evident (Fig. 1d). With both types of stimulation, addition of the same or a higher concentration of morphine to that still present in the bath did not depress the responses further. Washing out after prolonged exposure to morphine often produced a transient deepening of the depression.

After an effective dose, and apparently even after a small ineffective dose of morphine had been washed out of the bath, further addition of the drug within 90 min was without depressant effect on contractions evoked by transmural or by extrinsic nerve

stimulation, even in very large concentrations (up to 50 μg/ml). Fig. 3 illustrates the rapid development of tachyphylaxis to morphine. When tachyphylaxis had developed the contractions were often augmented by subsequent additions of morphine. Tachyphylaxis to morphine thus developed extremely rapidly in this tissue, in fact after a single exposure, and for this reason it was impossible to construct dose-response curves.

FIG. 3. Coaxial stimulation 2 Hz for 10 s every 60 s. At M, 5 μg/ml morphine; at D, 5 μg/ml diacetylmorphine; at Met, 5 μg/ml methadone; at E, 5 μg/ml etorphine. At W, the preparation was washed. Tachyphylaxis to morphine and cross tachyphylaxis to normally effective concentrations of diacetylmorphine and methadone developed rapidly. Only etorphine was powerful enough still to exert an effect.

Concentrations of about 50 μg/ml added when tachyphylaxis had developed often caused rhythmic contractions of the oesophagus followed by spasm.

Interaction with hexamethonium and mecamylamine

Hexamethonium, 8 μg/ml, and mecamylamine, 5 μg/ml, produced a 50–70% block of the contractions to extrinsic nerve stimulation in six preparations. Increase in the concentration did not produce a greater effect. In four preparations, hexamethonium was without depressant effect in all concentrations used (up to 100 μg/ml) and instead often augmented the contractions. The augmentation may have been due to its weak anticholinesterase activity (Schneider, 1966) or to an increase in acetylcholine release. Hexamethonium has been shown initially to increase acetylcholine release from the cat superior cervical ganglion (Matthews, 1966). The resistance of this preparation to ganglion blocking drugs has been noted before (Bowman & Everett, 1964). The ganglion blocking drugs produced a depression of responses to transmural stimulation also, but these responses could be restored to the control level by increasing the strength of the stimuli. Presumably responses to transmural stimulation in the absence of a ganglion blocking drug are partly due to stimulation of preganglionic fibres to ganglia within the tissue.

In the presence of concentrations of ganglion blocking drugs too low to depress the contractions by themselves, the depressant effect of morphine was augmented, and under these conditions there was little difference between the effect on responses evoked by extrinsic nerve stimulation or by transmural stimulation.

Effect on responses to acetylcholine and 5-hydroxytryptamine (5-HT)

At the height of the depressant effect of morphine on responses to electrical stimulation, contractions produced by acetylcholine (0·1–0·2 μg/ml) did not differ from the control responses produced before morphine was added. This result resembles that obtained with the guinea-pig ileum (Paton, 1957).

Morphine blocks responses of the ileum to 5-HT (Kosterlitz & Robinson, 1955; Gaddum & Picarelli, 1957), but in the chick oesophagus responses to 5-HT (1–5 ng/ml) were augmented during the depressant effect of morphine on the contractions evoked by electrical stimulation. The effects of diacetylmorphine, which were similar to

those of morphine, on responses to acetylcholine and 5-HT are illustrated in Fig. 4. Log dose-response curves to 5-HT were shifted to the left in the presence of morphine (5 μg/ml). The chick oesophagus was highly sensitive to 5-HT, especially in the summer months (May to September), concentrations as low as 0·5 ng/ml being effective in most preparations. The preparation was less sensitive in the winter months. Everett (1967) showed that the content of endogenous 5-HT in the chick ileum was lower in the summer than in the winter, and that the sensitivity of the crop to 5-HT was many times greater in the summer.

Responses of the oesophagus to 5-HT were blocked by phenoxybenzamine (1 μg/ml) indicating that the receptors involved, according to the classification of Gaddum & Picarelli (1957), are of the "D" type, rather than of the "M" type. Phenoxybenzamine also blocked responses to acetylcholine illustrating its known atropine-like action.

Methysergide (1·0–2·5 μg/ml) blocked contractions produced by 5-HT, and this blockade persisted for the remainder of the experiment despite repeated washing. When the stimulant action of 5-HT was blocked by methysergide, larger concentra-

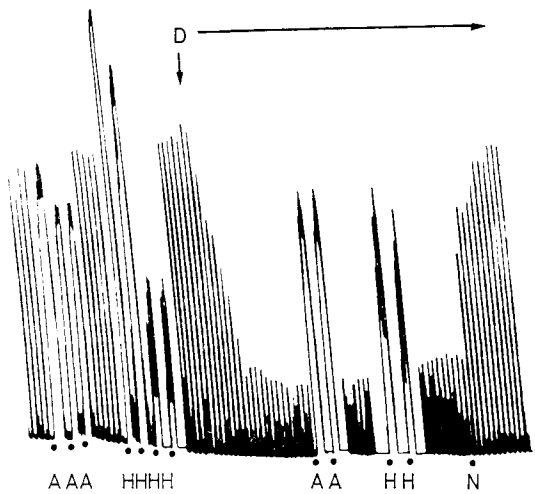

FIG. 4. Coaxial stimulation. 2 Hz for 10 s every 60 s. At A, 0·01 μg/ml acetylcholine. At H, 5-hydroxytryptamine; the first two of the group of four response to 5-HT are to 0·05 μg/ml and 0·025 μg/ml respectively; all remaining responses to 5-HT are to 0·005 μg/ml. From D to the end of the experiment the Krebs solution was changed to one containing 5 μg/ml diacetylmorphine. At N, 5 μg/ml nalorphine. Diacetylmorphine depressed the electrically evoked contractions, left unaffected those produced by acetylcholine and augmented those produced by 5-HT. Nalorphine reversed the depressant effect of diacetylmorphine in its continued presence.

tions of 5-HT (0·5–5 μg/ml) depressed responses of the oesophagus to nerve stimulation. With repeated large doses of 5-HT, tachyphylaxis to its depressant effect developed. Morphine did not alter the depressant effect of 5-HT nor change the rate of development of tachyphylaxis to it. Large doses of methysergide (2·5–5 μg/ml) themselves depressed the responses to electrical stimulation, but this effect was readily reversed on washing the tissue. Methysergide was without effect on responses to acetylcholine or histamine.

Other analgesics

The narcotic analgesics, etorphine, diacetylmorphine, methadone, pethidine, propoxyphene, pentazocine, cyclazocine and phenazocine, were tested, each on at least 8 different preparations in concentrations ranging from 0·1–10 μg/ml. Etorphine was

the most potent compound tested, and in concentrations of 1 μg/ml and above, depressed the contractions evoked by electrical stimulation to about the same extent as did 5 μg/ml morphine. Although etorphine was the most potent compound, it was relatively very much less potent than it is in the guinea-pig ileum, or as an analgesic in mammalian species (Cox & Weinstock, 1966).

Etorphine, the only other drug tested during alternate extrinsic nerve and coaxial stimulation,behaved like morphine in that it was more effective in depressing responses to coaxial stimulation (Fig. 2b). The remaining drugs were effective in depressing the contractions in concentrations of 5 μg/ml. Etorphine and diacetylmorphine possessed a more rapid onset of action than all other drugs including morphine, whereas methadone, pethidine, pentazocine, phenazocine, propoxyphene and cyclazocine were slower in onset of action than was morphine. With methadone, pentazocine, phenazocine and cyclazocine the depression was often preceded by a small augmentation of contractions, and this was particularly evident with cyclazocine. With the exceptions of pethidine, propoxyphene and pentazocine, tachyphylaxis to all of the drugs developed rapidly after a single application, and cross-tolerance between drugs was also immediately evident (Fig. 3). The higher potency of etorphine relative to the other drugs was often evident in experiments in which complete tolerance to other drugs had been induced. Under these conditions, etorphine often continued to produce an obvious depressant effect (Fig. 3). However, this difference was merely quantitative. More pronounced tolerance induced by higher doses of morphine was also transferred to etorphine. Because of the rapidly developing tachyphylaxis, it was impossible to determine relative potency or to construct dose-response curves. Pethidine, propoxyphene and pentazocine continued to produce a depressant effect with successive additions, and when complete tolerance to another drug had developed, these three drugs remained effective to some extent.

When the drugs were left in contact with the tissue for 30 min, full recovery to control amplitude, accompanied by irregular contractions of the preparation, occurred within 20 min in the case of etorphine and diacetylmorphine. Partial recovery accompanied by irregular contractions occurred with methadone and pethidine, but with pentazocine, phenazocine, propoxyphene and cyclazocine the depression of contractions was maintained. Irregular contractions, unrelated to the electrical stimulation, occurred with pentazocine, phenazocine, and propoxyphene, but not with cyclazocine.

On washing out the drug after prolonged exposure, complete and rapid recovery followed by augmentation above control level occurred with cyclazocine. With pentazocine, propoxyphene and phenazocine, on the other hand, recovery was slow after washing and often a temporary deepening of the depression intervened before the onset of a slow recovery. Recovery following washout of etorphine, diacetylmorphine, methadone, and pethidine was intermediate in rate; in the case of etorphine, the oesophagus often went into a temporary spasm on washing out.

With all the drugs, contractions evoked at lower frequencies of stimulation were more affected than those evoked by higher frequencies. Fig. 1b and c illustrate these effects of etorphine and pethidine at different frequencies of stimulation.

Methadone, diacetylmorphine, etorphine, cyclazocine and phenazocine, like morphine, were without effect on responses of the oesophagus to acetylcholine. The effects of diacetylmorphine are illustrated in Fig. 4. However, pethidine, propoxyphene and pentazocine depressed responses to acetylcholine.

With the exception of methadone, all of the compounds (diacetylmorphine, etorphine, pethidine, propoxyphene, pentazocine, cyclazocine and phenazocine) potentiated 5-HT in its contractor action on the oesophagus. Fig. 4 illustrates this effect of diacetylmorphine. Methadone was without effect on responses to 5-HT.

Nalorphine

Nalorphine slightly augmented the contractions of the chick oesophagus in all effective concentrations (1 μg/ml and above). There was no depressant effect like that seen in the guinea-pig ileum (Paton, 1957). Small concentrations (below 0·5 μg/ml) added during morphine depression, enhanced the depressant effect (Fig. 5a) but with

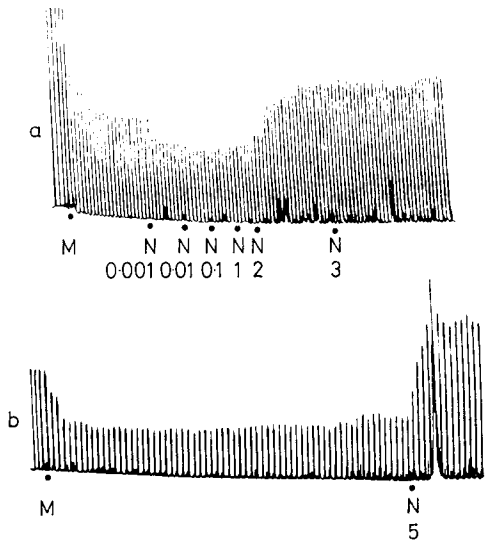

FIG. 5. a and b are from different experiments. Coaxial stimulation. 2 Hz for 10 s every 60 s. At M, 5 μg/ml morphine. At N, nalorphine. The concentrations of nalorphine added are given in μg/ml. a. Small concentrations of nalorphine (0·001–0·1 μg/ml) depressed the contractions, and antagonism was incomplete even when the cumulative concentration exceeded 5 μg/ml. b. 5 μg/ml nalorphine as a single addition antagonized morphine depression and augmented the contractions above control.

larger concentrations a clear antagonism of morphine depression was evident (Fig. 5b). If the concentration of nalorphine was gradually built up by cumulative addition, the antagonism of morphine was never complete (Fig. 5a). However, when 5 μg/ml of nalorphine was added as a single dose, antagonism was rapid and complete, the contractions becoming augmented above the control level (Fig. 5b).

Morphine was ineffective in depressing the contractions when nalorphine had been previously added.

Nalorphine (5 μg/ml) also completely antagonized the depressant effects of diacetylmorphine (Fig. 4), etorphine (Fig. 2), methadone and phenazocine. Pethidine and propoxyphene were only partially antagonized, and pentazocine and cyclazocine were not antagonized.

DISCUSSION

The results showed that morphine and other narcotic analgesics depressed contractions of the chick oesophagus evoked through stimulation of its cholinergic nerves, as they do those of the guinea-pig ileum. However, the chick preparation was much

less sensitive to the depressant effects of the analgesics than is the guinea-pig ileum, and tachyphylaxis to their effects and cross tolerance between the different drugs developed much more rapidly.

Responses evoked by transmural stimulation were more depressed by morphine and etorphine than were those evoked by extrinsic nerve stimulation at the same frequency. This is despite the fact that the nervous elements excited by transmural stimulation must be, or at least must include, the final common path for both types of stimulation. A possible explanation is that morphine and etorphine have an opposing stimulant action at a site more central than the intramuscular nerve endings, probably the parasympathetic ganglia. The observation that hexamethonium and mecamylamine potentiated morphine's depressant effect on contractions, especially those evoked by extrinsic nerve stimulation, and removed the difference between the two types of stimulation, supports the idea that morphine stimulates the ganglia and suggests that acetylcholine receptors are involved. It is unlikely that morphine itself stimulates acetylcholine receptors directly, and an indirect mechanism is therefore more probable. Morphine is known to possess anticholinesterase activity (Randall, Kruger & others, 1953) and this action may account for the effect. It is of interest that mecamylamine has been shown to potentiate the analgesic action of morphine in rats (Gupta & Dhawan, 1961) although this action may be irrelevant to the interaction in the oesophagus.

As in the guinea-pig ileum, the site of the depressant actions of morphine and of diacetylmorphine, methadone, cyclazocine, phenazocine and etorphine appeared to be pre-junctional since the responses to acetylcholine were not depressed. The depression of neurally evoked contractions was never complete showing that there is a morphine-resistant as well as a morphine-sensitive fraction of releasable acetylcholine, the former playing a relatively greater role in contractions evoked at higher frequencies of nerve stimulation. Paton (1969) has discussed his similar findings in the guinea-pig ileum. Pethidine, known to possess atropine-like activity (Paton, 1957; Fennessy & others, 1969), depressed responses to acetylcholine, as also did propoxyphene and pentazocine. The atropine-like action of these three drugs probably accounts for the fact that they continued to exert a depressant effect on contractions of the oesophagus when complete tolerance had been produced to other analgesics.

The inability of morphine and other analgesics to block responses to 5-HT indicates that, in the chick oesophagus, 5-HT acts directly on the smooth muscle rather than via a nervous mechanism as is believed to be the case in the guinea-pig ileum (Robertson, 1953; Rocha e Silva, Valle & Picarelli, 1953; Gaddum & Hameed, 1954). In preparations of alimentary canal from other species, both "M" and "D" types of 5-HT receptor are believed to be located in the nervous elements (Brownlee & Johnson, 1963; Day & Vane, 1963; Harry, 1963). With the exception of methadone, which was without effect, the analgesics in fact potentiated 5-HT in its ability to cause contraction of the chick oesophagus. This could be explained if 5-HT also possessed an opposing, but masked, inhibitory action that was blocked by the analgesics. A depressant action of higher concentrations of 5-HT on neurally evoked contractions could be demonstrated after blockade of its stimulant action by methysergide. However, morphine did not modify this depressant action indicating that potentiation of the contractor action of 5-HT by morphine cannot be explained in this way.

In the guinea-pig ileum, nalorphine produces a depressant effect resembling that of morphine and it is difficult to demonstrate any antagonism between the two drugs

(Paton, 1957; Gyang & Kosterlitz, 1966; Fennessy & others, 1969), although some degree of antagonism has been demonstrated with low concentrations of nalorphine (Cox & Weinstock, 1966). In contrast, nalorphine enhanced the contractions of the chick preparation, and antagonism to most of the analgesics was readily demonstrated. However, results to be reported indicate that the effect of nalorphine is nonspecific in this tissue, since depression of contractions produced by a wide range of unrelated substances are also antagonized by it.

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