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The depressant action of morphine on transmission at a skeletal neuromuscular junction is non-specific

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The depressant actions of apomorphine, etorphine, dextromoramide, laevomoramide, naloxone, codeine, morphine and nalorphine have been examined on the rat diaphragm preparation. Their descending order of potency (in the order given above) differed greatly from that published for activity at specific opiate receptors. The depressant action of morphine was not antagonized by naloxone. The stereoisomers dextro- and laevomoramide were equipotent in depressing the preparation. On the transmurally stimulated guinea-pig ileum preparation the depressant action of dextromoramide was antagonized by naloxone. Laevomoramide was 10 000 times less potent than its (+)-isomer, and was not antagonized by naloxone. It is concluded that the effects of narcotic analgesics on transmission at a skeletal neuromuscular junction are not mediated via opiate receptors.

The depressant action of morphine and its pharmacological analogues upon transmission at some postganglionic parasympathetic nerve endings has been the subject of much investigation (Lees, Kosterlitz & Waterfield, 1972, and references cited therein). At these sites morphine depresses the release of acetylcholine. Since potency ratios between morphine and its pharmacological analogues at this peripheral site are similar to those for their central depressant actions (Harris, 1970), the peripheral site has often been used as a model.

In addition to this specific depressant action on acetylcholine release, morphine also has a non-specific depressant action which is seen at higher concentrations. The differences between the characteristics of morphine's specific and non-specific actions have been summarized by Lees & others (1972). The main characteristics of its specific action are that it is apparent at low concentrations, at low frequencies of stimulation, and is competitively antagonized by naloxone.

In 1971 Frederickson & Pinsky and Pinsky & Frederickson reported that morphine also depresses the release of acetylcholine at the skeletal neuromuscular junction, and suggested that this site provided a simple alternative model for examinations of the mechanism of morphine's action.

In view of the high concentrations used by these authors it seemed possible that this effect was non-specific. We have therefore re-examined the characteristics of the depressant action of morphine at a skeletal neuromuscular junction, and applied three tests to determine whether the action is specific or not. Firstly we have examined the order of potency of a variety of morphine analogues, and compared them with published orders of potency for their specific actions on pain perception, and upon depression of the transmurally stimulated ileum of the guinea-pig. Secondly we have examined whether the action of morphine could be antagonized by naloxone.

Thirdly we have determined the potency of the two stereoisomers of a narcotic analgesic in only one of which resides specific morphine-like activity.

METHODS

The rat diaphragm preparation. Male or female Wistar rats were used. Hemidiaphragms were suspended in a 50 ml tissue bath containing Krebs solution bubbled with 5% CO_2 in oxygen and maintained at 36°. Electrodes were positioned to permit either stimulation via the phrenic nerve (indirect), or direct to the muscle. For nerve stimulation twitches were elicited at supramaximal voltage (usually 15–20 V), at a pulse width of 0·1 ms and a frequency of 0·2 Hz. For direct stimulation the pulse width was increased to 5 ms, and the voltage to 50 V. Examinations of the effects of narcotic agonists upon directly elicited twitches were done in the presence of 10 μM tubocurarine. Drugs were added to the tissue bath in volumes of less than 2·5 ml, or when solubility precluded this, by total exchange of tissue bath fluid. Unless otherwise stated drug contact time was 3 min.

The transmurally stimulated guinea-pig ileum preparation. Sections of ileum (3 cm) were suspended in a 20 ml tissue bath containing Krebs solution bubbled with 5% CO_2 in oxygen and maintained at 38°. The preparation was stimulated transmurally by a pair of stainless steel electrodes, one of which was situated in the lumen. Pulses at supramaximal voltage (usually 20 V), pulse width 0·3 ms and frequency 0·33 Hz, were used. Drugs were left in contact with the preparation until equilibrium was reached or spontaneous recovery occurred. Because of the considerable speed with which tachyphylaxis developed to the depressant action of the narcotic agonists, a fresh section of ileum was used for each drug addition. To minimize bias due to any regional differences in sensitivity of the ileum to narcotic agonists, the order in which sections of ileum were cut from the whole length was randomized, as was the order in which drug concentrations were examined.

Drugs. Drugs used were atropine sulphate (BDH); apomorphine hydrochloride (Wander); codeine hydrochloride (Roche); dextromoramide tartrate (M.C.P. Pure Drugs Ltd); etorphine hydrochloride (Reckitt & Colman); gallamine triethiode (May & Baker); laevomoramide (ACF Chemiefarma); morphine hydrochloride (Macfarlan & Smith); nalorphine hydrobromide (Burroughs Wellcome); naloxone hydrochloride (Endo Laboratories Inc.); tubocurarine hydrochloride (BDH).

Laevomoramide tartrate was prepared from the free base by addition of solutions of laevomoramide and D-(+)-tartaric acid in acetone. The salt was obtained by evaporation, and confirmed as being optically pure by comparison with dextromoramide tartrate using a Thorne-Bendix automatic polarimeter.

Drugs were dissolved in water or in the Krebs solution of the preheating column except apomorphine which was made up in 0·02% sodium metabisulphite.

RESULTS

The effects of narcotic agonists on directly and indirectly elicited contractions of the rat diaphragm preparation. The effects of codeine, dextromoramide, etorphine, morphine, nalorphine and naloxone were examined. For some of the drugs used the log concentration effect lines had to be constructed from single experiments because extremely high concentrations were required to produce any effect and only limited quantities were available.

All the compounds shared a qualitatively similar action with four phases. Firstly there was an initial increase in indirect twitch height. This was followed by selective depression of indirect twitches without effect on directly elicited contractions. At higher concentrations there was also depression of direct twitches. With some drugs this phase was associated with an increase in the resting tension of the preparation.

Quantitatively the compounds differed not only in potency but also in the incidence of the various phases referred to above. For instance nalorphine caused the most marked twitch potentiation, dextromoramide and morphine the least. Increases in resting tension were rarely seen with codeine, but marked increases occurred with etorphine and nalorphine.

Log concentration effect lines for the narcotic agonists are compared with those of tubocurarine and gallamine (as conventional neuromuscular blocking agents) and apomorphine (as a non-narcotic base) in Fig. 1.

The narcotic agonists ranged in potency from the most potent, dextromoramide, (active at a concentration of 30μM), to the least potent, nalorphine (active at 10 mM). The descending order of potency (at the ID20) was etorphine, dextromoramide, naloxone, codeine, morphine and nalorphine.

The effects of two non-narcotic bases, atropine and apomorphine, were also examined. Atropine had effects qualitatively and quantitatively similar to morphine, causing selective depression of indirect contractions followed at higher concentrations by an increase in resting tension, and depression of direct twitches. Its log concentration effect line (not in Fig. 1 for clarity) falls close to that of morphine.

Apomorphine also depressed indirect twitches without initial effect upon direct twitches. Of the compounds tested not normally associated as having an action at the neuromuscular junction, apomorphine was the most potent.

Interactions between morphine and naloxone. In no instance did the specific narcotic antagonist naloxone antagonize the twitch depressant action of morphine—

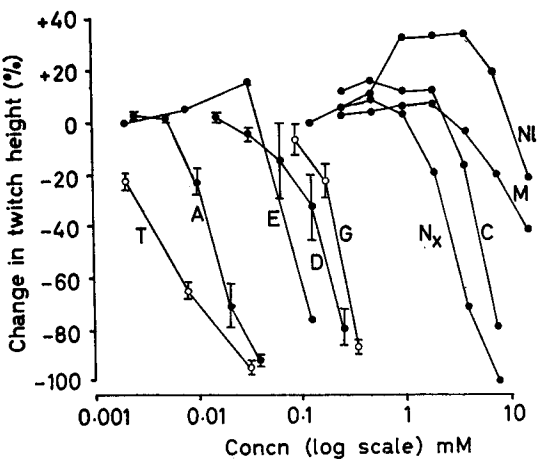

FIG. 1. The relation between the concentrations of various drugs and the percentage changes in contraction of the indirectly stimulated rat diaphragm preparation. The drugs were: (+)- tubocurarine (T), apomorphine (A), etorphine (E), dextromoramide (D), gallamine (G), naloxone (Nx), codeine (C), morphine (M), nalorphine (Nl). Vertical bars (where included) are ± s.e.

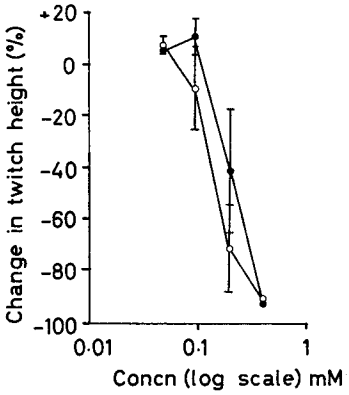

FIG. 2. Cumulative log concentration effect lines for the depressant actions of dextromoramide (open circles) and laevomoramide (closed circles) on the indirectly stimulated rat diaphragm preparation. Each point is the mean of 4 determinations (±s.e.).

indeed, as may be seen from Fig. 1, naloxone was itself a more potent depressant than was morphine.

When concentrations of naloxone having no twitch depressant action were added with morphine, the net result had the characteristics of synergism. For instance 1 mM naloxone was virtually without effect when given alone. When added with 10 mM morphine—a concentration which normally depressed indirect twitches by 22·2 ± 2·0%, the net depression was 34·5 ± 5·0%.

1 mM naloxone also augmented the depressant action of 10 mM nalorphine.

A comparison between dextromoramide and laevomoramide on the rat diaphragm

In this part of the work the method was changed. With both isomers it was found that highly variable results were obtained when the effects of a given concentration were re-examined on the preparation, even when the interval between additions was greater than 30 min. For instance an ID20 of dextromoramide repeated on the same preparation could cause anything up to 100% inhibition. Because of the expense and inconvenience of limiting one drug addition to one preparation, and in view of the fact that the purpose of the experiment was to compare the two isomers, cumulative log concentration effect lines were established.

These are shown in Fig. 2. There was no significant difference between the potency of the two isomers in depressing neuromuscular transmission, nor was there any significant difference between the time interval between drug additions and the time when 'equilibrium' occurred.

The effects of dextromoramide and laevomoramide on the transmurally stimulated guinea-pig ileum

Although laevomoramide has been reported to possess no narcotic analgesic activity (Janssen & Jageneau, 1957), it was thought necessary to confirm the actions of dextro- and laevomoramide at the specific narcotic receptor of the ileum.

Fig. 3 shows the concentration effect lines for dextromoramide and laevomoramide alone and in the presence of 20 nM naloxone. Dextromoramide was found to be a potent depressant of the transmurally stimulated ileum with an ID50 of about 4 nM comparing reasonably with the 6·7 nM found by Kosterlitz & Watt (1968) on the same preparation. In the presence of 20 nM naloxone the line was shifted to the right without deviation from parallelism.

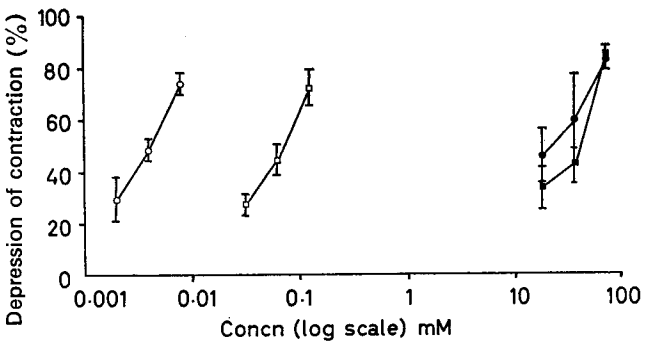

FIG. 3. The depressant actions of dextromoramide (open symbols) and laevomoramide (closed symbols), alone (circles) and in the presence of 20 nM naloxone (squares) on the transmurally stimulated guinea-pig ileum. Each point is the mean of 4 determinations (± s.e.).

On the other hand laevomoramide was a weak depressant, being over 10 000 times less potent than the (+)-isomer. When the effects of laevomoramide were re-examined in the presence of 20 nM naloxone there were no significant differences from the responses in the absence of naloxone.

Apomorphine was found to be a weak depressant of the transmurally stimulated ileum with an ID50 of 1·7 μM. In the presence of 20 nM naloxone the log concentration was slightly less steep but this was not significant.

DISCUSSION

The actions of the narcotic analgesics at the skeletal neuromuscular junction fulfilled none of the three criteria for specificity applied.

The most crucial evidence is the complete absence of any antagonism of morphine or nalorphine by naloxone.

It is a characteristic of structurally specific agents that they demonstrate stereospecificity. The impressive stereospecificity of a typical narcotic analgesic—dextromoramide—is confirmed on the guinea-pig ileum. The fact that at the skeletal neuromuscular junction the two isomers are equipotent is additional evidence of the lack of specificity at this site. This observation is compatible with that of Simon & Rosenberg (1970) who showed that on the squid giant axon both levorphanol and its dextrorotatory enantiomer were equipotent as depressants of axonal conduction despite the fact that the latter is virtually devoid of analgesic activity.

Potency ratios of narcotic agonists at one specific site of action bear a close resemblance to those seen at other specific sites. For instance, it is well known that potency ratios of narcotic agonists on the transmurally stimulated guinea-pig ileum are similar to those for analgesia in the experimental animal and in man (Gyang & Kosterlitz, 1966; Cox & Weinstock, 1966; Harris, 1970). Table 1 compares the potency ratios of some morphine derivatives on the transmurally stimulated guinea-pig ileum and on the rat diaphragm preparation. There is no significant correlation between them ($r = -0{\cdot}19$, $P > 0{\cdot}6$). The most notable anomalies are the potency of naloxone at the neuromuscular junction (no ID50 could be determined for it on the guinea-pig ileum, Kosterlitz & Watt, 1968) and codeine, which is more potent than morphine at the neuromuscular junction, yet is some 150 times weaker than morphine at its specific receptor. Although this lack of correlation between the potencies of

Table 1. *A comparison of the ratio of concentrations of drugs (with reference to morphine = 1)* to cause a 50% inhibition of the transmurally stimulated guinea-pig ileum, and a 20% inhibition of the rat diaphragm preparation.*

	Guinea-pig ileum**	Rat diaphragm
Etorphine	0·0013	0·0069
Dextromoramide	0·098	0·0096
Nalorphine	0·34	1·95
Morphine	1·00	1·00
Apomorphine	46·37	0·0011
Codeine	151·03	0·54
Naloxone	>997·07	0·26

* ID50 (guinea-pig ileum) for morphine = 68·2 nM (Kosterlitz & Watt, 1968); ID20 (rat diaphragm) for morphine = 7·76 mM.

** Ratios include those taken from Kosterlitz & Watt (1968), and Cox & Weinstock (1966).

morphine and naloxone on the rat diaphragm preparation and at the specific narcotic receptor was in evidence, the relative potencies correlate well with those described by Soteropoulos & Standaert (1972) on the cat soleus neuromuscular preparation.

Whilst it may be concluded that the actions of the narcotic analgesics at the skeletal neuromuscular junction are non-specific, some discussion is necessary about their possible mechanism of action.

Ignoring apomorphine, the two most potent compounds not normally associated with any action at the neuromuscular junction are dextromoramide and etorphine. Both these compounds are relatively lipid soluble, whilst morphine, nalorphine, naloxone and, to a lesser extent, codeine are more polar. There is thus some suggestion of a correlation between activity and lipid solubility. This could enable the compounds to gain access to a lipid phase to produce a non-specific depression.

That morphine depresses the release of acetylcholine at the motor nerve ending was convincingly shown by Frederickson & Pinsky (1971). These authors dismissed local anaesthetic action as the cause of this depression using as evidence the work of Thompson (quoted in Paton, 1957), who showed that in concentrations up to 10 mM morphine had no effect upon conduction in the frog sciatic nerve.

However, other authors have suggested a local anaesthetic action of narcotic analgesics in high concentrations on the squid giant axon (Simon & Rosenberg, 1970; Frazier, Murayama & others, 1972), and on the Renshaw cell (Duggan & Curtis, 1972).

We therefore conclude from our evidence and from that of others that the action of morphine and its pharmacological analogues at the skeletal neuromuscular junction is non-specific, and that this peripheral site of action may be unhelpful as an alternative model for investigations of the molecular basis of morphine's action.

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