NEUROMUSCULAR EFFECTS OF SOME OPIOID AGONISTS AND ANTAGONISTS*

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Abstract—The effects of morphine, pethidine, (−)naloxone, N-methylnaloxone, naltraxone and levallorphan on acetylcholine-induced contraction of the toad rectus were studied. The drugs were shown to inhibit the contraction, and their inhibitory effect was suggested to be partly mediated via a peripheral opiate binding site. The depression of acetylcholine-induced contraction by levallorphan and dextrallorphan might indicate possible involvement of stereospecific binding sites, as the latter required a significantly higher concentration to produce the same magnitude of depression. Statistical analysis of the slope of the computer-plotted dose-response of acetylcholine in the presence and absence of each of the opioids indicates that these drugs can be classified into four categories. Morphine and naltrexone each formed a class of its own; (−)naloxone, N-methylnaloxone and pethidine formed another class; levallorphan and dextrallorphan formed the fourth class. The classification of the opioids into four categories reveals the possible existence of multiple opiate binding sites on the skeletal muscle. The significance of each of the sub-types of binding sites in the contraction of skeletal muscle and the mechanism by which it affects the contraction remains to be investigated.

Of late, the neuromuscular action of morphine has been of interest to pharmacologists. At concentrations of 10⁻⁸ to 10⁻⁵ M, the drug has been found to depress cholinergic transmission in the smooth muscle of experimental animals (1–5). At higher concentrations (10⁻³ to 10⁻⁵ M), the same effect was also observed in the skeletal muscle (6–8). Frank (9) suggested the existence of opiate receptors in the skeletal muscle of the frog. From his work on frog’s skeletal muscle (10) and sciatic nerve (11), he attributed the marked difference in the effective concentrations of morphine in the two type of muscular preparations to the difference in sensitivity of the receptors to the opioid (12). However, whether the depressant effect of opiate drugs on neuromuscular transmission involved the mediation of stereospecific sites or not is still controversial (8, 13, 14). We attempt to address this problem by studying the effect of opiate agonists, antagonists, stereoisomer of one of the antagonists and N-methylnaloxone on the Ach-induced contractions of the toad rectus abdominis.

Materials and Methods

Rectus muscle preparation: The rectus muscle from toads weighing between 30 to 40 g was used. The tissue of approximately 3 cm length was mounted and stretched with a weight of 10 g in a 10 ml organ bath at room temperature (28–30°C) containing Frog-Ringer solution bubbled with air. It was allowed to equilibrate for 1 hr before drug
exposure. During the equilibration period, the bath solution was replaced frequently. Contractile responses were recorded on a Washington Oscillograph recorder (Model 400 MD1) through an isotonic lever transducer (Model T2) and a strain gauge coupler (Model FC117).

The responses of the rectus to 4, 8, 16, 32×10⁻⁶ M of acetylcholine in the absence and presence of each increasing concentration (10, 20, 40, 80, 160, 320×10⁻⁶ M) of each opioid were determined. The bracketing technique was employed. Following each Ach-induced response, the organ bath was drained, and the preparation was washed three times with 10 ml of the Ringer. In all cases, the recovery of the responses after washing was more than 95%. The pH of the Frog Ringer was 6.5, and dissolutions of the drugs even at concentrations 320×10⁻⁶ M did not alter the pH of this solution. For the Ringer containing Ach or Ach plus the opioid, the contact time with the preparation was one min. Prior to exposure to the Ringer containing Ach plus the respective opioid, the preparation was exposed to the Ringer containing the corresponding opioid for 2 min. Similarly, 2 min exposure to plain Ringer was employed before adding the Ringer containing Ach. For each set of dose-response experiments, the rectus muscle was alternately exposed to increasing concentration of Ach and the corresponding combination of Ach and one of the doses of opioid. For a dose of each opioid, five or six pairs of dose-response relations were obtained using five or six rectus muscles.

Drugs: The following drugs were used: Acetylcholine iodide (BDH chemicals, England), morphine hydrochloride (United Pharmaceutical Works Ltd., Holland), pethidine hydrochloride (Govt. Pharm. Laboratory, Singapore), (−)-naloxone hydrochloride and naltrexone hydrochloride (gift from Endo Laboratories, U.S.A.), N-methyl-
naloxone hydrobromide [(MRZ 2593) gift from Dr. H. Merz, Boehringer Ingelheim, F.R.G.], levallorphan tartrate and dextrallorphan hydrobromide (gift from Dr. W. Haefely, Hoffmann-La Roche Ltd., Switzerland).

Statistical analysis: The dose-response relations were linearly plotted, and the responses at 16×10⁻⁶ M of Ach were predicted using the regression lines. The difference in these predicted responses in the absence and presence of opioid was noted for each of the different concentrations of the opioid used. The experiment was replicated five or six times for each concentration of the opioid, and hence, five or six sets of differences were available for each concentration. When regressed against the concentrations of the opioids, the replicated differences provided a comparison between opioids for slope and position of the regression lines. All statistical calculations were carried out on a computer (TRS-80 model II).

Results

There was a general trend that the opioid agonists (morphine and pethidine) and antagonists (naloxone, N-methylnaloxone, naltrexone and levallorphan) produced an inhibition of Ach-induced contractions of the toad rectus in a competitive manner. Morphine did not produce a dose-dependent inhibition. Table 1 shows the averaged differences in response height (obtained at 16×10⁻⁶ M Ach in the absence and presence of each opioid) and the varying concentrations of the opioids. For each opioid, a linear regression of differences in heights against concentration of the opioid used was carried out. The different regression lines were then compared for differences in slope and position as shown in Fig. 1.

The slopes of the regression lines were compared using the t-test. Table 2 gives a matrix of the t-values obtained. It can be seen
that morphine and (-)naltrexone each forms a class of its own, the slopes of their regression lines being significantly different from the rest of the opioids. Among the other opioids, levallorphan and dextrallorphan did not significantly differ in the slopes of their regression lines. However, when tested for position, the pair of regression lines for levallorphan and dextrallorphan was found to be significantly different (t=13.8; d.f.=56), viz., each unit increase in concentration of the opioid produced different magnitudes of responses. On the other hand, (-)naloxone was found to be grouped with N-methylnaloxone and pethidine. Interestingly, the regression lines obtained for N-methylnaloxone and pethidine did not differ from each other either in slope or position (t=0.04; d.f.=69). It appeared, therefore, that these opioids produced the same magnitude of depressant effect on the Ach-induced contractions.

Table 1. Averaged differences in response height and varying concentrations of opioids

| Type of opioid     | Opioid concentrations $\times 10^{-6}$ M |
|--------------------|------------------------------------------|
|                    | 10       | 20       | 40       | 80       | 160      | 320      |
| (-) Naloxone       |          |          |          |          |          |          |
| S.D.               | 0.05     | 0.02     | 0.34     | -0.31    | -0.55    | -0.66    |
| N-methylnaloxone   | -0.23    | -0.27    | 0.09     | -0.36    | -0.50    | -0.77    |
| (MRZ 2593)         |          |          |          |          |          |          |
| S.D.               | 0.12     | 0.16     | 0.24     | 0.13     | 0.08     | 0.18     |
| Naltrexone         | -0.11    | -0.19    | -0.15    | -0.24    | -0.23    | -0.47    |
| S.D.               | 0.05     | 0.09     | 0.04     | 0.08     | 0.07     | 0.25     |
| Morphine           | -0.05    | -0.18    | -0.05    | -0.18    | 0.02     | -0.03    |
| S.D.               | 0.10     | 0.07     | 0.08     | 0.19     | 0.24     | 0.12     |
| Pethidine          | -0.13    | -0.19    | -0.15    | -0.20    | -0.60    | -0.79    |
| S.D.               | 0.17     | 0.17     | 0.04     | 0.13     | 0.17     | 0.28     |
| Levallorphan       | 0.02     | 0.15     | -0.76    | -0.54    | -0.97    | N.T.     |
| S.D.               | 0.17     | 0.04     | 0.60     | 0.20     | 0.66     |          |
| Dextrallorphan     | -0.02    | -0.02    | -0.30    | -0.55    | -0.58    | N.T.     |
| S.D.               | 0.07     | 0.03     | 0.20     | 0.20     | 0.17     |          |

S.D.= Standard deviation, N.T.=Not tested. The negative sign indicates inhibition of the Ach-induced contraction. For each concentration of opioid 5 or 6 preparations were used.

Fig. 1. Regression lines of differences in responses (height) against concentrations of opioids. ○ MOR: Morphine, × NLX: Naltrexone, □ NAL=(-) Naloxone, ● PET: Pethidine, ■ MRZ=N-methylnaloxone, △ DEX=Dextrallorphan, Δ LEV: Levallorphan

Discussion

The results obtained clearly show that both the opioid agonist (pethidine) and antagonists [(-)naloxone, N-methylnaloxone, naltrexone and levallorphan] in adequate concentrations can inhibit acetylcholine-induced contractions of the toad rectus. The inhibitory
Table 2. Matrix of $t$-values of readings obtained from the comparison of slopes of regression lines for pairs of opioids

|                | (−) Naloxone | MRZ 2593 | Naltrexone | Morphine | Pethidine | Levallophan | Dextrallorphan |
|----------------|--------------|----------|------------|----------|-----------|-------------|---------------|
| (−) Naloxone   |              |          |            |          |           |             |               |
| MRZ 2593       | 1.3 (68)     | N.S.     |            |          |           |             |               |
| Naltrexone     | −3.9 (68)    | −2.8 (68)|            | P<0.01   | P<0.01    |             |               |
| Morphine       | −6.4 (65)    | −5.9 (65)| −4.4 (65)  | P<0.001  | P<0.001   | P<0.001     |               |
| Pethidine      | −1.0 (68)    | 0.5 (68) | 4.0 (68)   | P<0.001  | P<0.001   | P<0.001     |               |
| Levallophan    | 2.7 (61)     | 3.3 (61) | 4.4 (61)   | 5.2 (68) | 3.3 (61)  | 3.3 (61)    | 5.2 (68)      |
| Dextrallorphan | 1.4 (62)     | 2.6 (62) | 6.6 (59)   | 2.5 (62) | 2.5 (62)  | −1.5 (55)   | N.S.          |

Values in parentheses indicate the degrees of freedom for the $t$-test. N.S. = Not significant.
effect of the opioid agonists might be partly mediated via a peripheral opiate binding site. This proposal is in agreement with the results of other investigators which showed that morphine and pethidine blocked the muscle action potential in frog's sartorius muscle fibers (9, 15) and that pethidine depressed the twitch response to nerve stimulation in frog sciatic nerve-sartorius muscle preparation (12). However, it is not known at present whether the depressant action of morphine and other opioid agonists involve stereospecific binding sites. This aspect requires further analysis with stereoisomers such as levorphanol and dextrorphan and /-methadone and d-methadone.

On the other hand, high concentrations of the opioid antagonists such as (−)naloxone, N-methyl-naloxone, naltrexone and levallorphan also produced inhibition of acetylcholine-induced responses. This might be partly due to the phenomenon of the "opiod-binding site mediated event", as high concentrations of opiate antagonists are known to produce morphine-like agonist effects (16–23). Non-opioid (8, 18, 24) or naloxone-insensitive receptor mechanisms (12) have also been suggested to contribute to the depression of Ach-induced contraction. Whether the depressive effect of the opioid antagonists on acetylcholine-induced responses involves end plate action potential, muscle action potential and excitation-contraction coupling are yet to be studied. The depression of acetylcholine-induced contraction by the isomeric pair of the opioid antagonists, levallorphan and dextrallorphan, might indicate the possible involvement of stereospecific binding sites as dextrallorphan, the (+)enantiomer required a significantly higher concentration to produce the same magnitude of depression.

Lastly, the classification of the opioids into four categories based on the slope of the regression lines reveal the possible existence of multiple opiate binding sites on the skeletal muscle. The significance of each of the sub-types of binding sites and the mechanism by which it affects the contraction remains to be investigated.

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