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Effect of naloxone on the sensitivity of the vas deferens to various agonists

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The effect of naloxone in vitro, on noradrenaline (NA)-induced responses in guinea-pig isolated vas deferens was studied. The responses of NA were potentiated by naloxone (3 μM). It potentiated the responses to methoxamine which has least affinity for uptake sites. Responses induced by acetylcholine and potassium chloride were also potentiated by naloxone. Low calcium (0·8 mM) in the medium antagonized the potentiating action of naloxone. It is suggested that naloxone produces non-specific potentiation, by promoting calcium influx at the post-synaptic site.

Antagonism of various agonists by naloxone has been reported by Lee & Berkowitz (1976) using the rat aortic spiral strip preparation, and this antagonism was shown to be dependent on Ca^{2+} concentration. The present study was undertaken to investigate the effect of naloxone on the sensitivity of the guinea-pig isolated vas deferens preparation to noradrenaline and to other agonists. This preparation does not possess opiate receptors (Johnson et al 1978). The possible involvement of Ca^{2+} concentration was also examined.

MATERIALS AND METHODS

Male guinea-pigs, 200–250 g were killed by a blow on the head. Vasa deferentia were dissected out and mounted in a 30 ml organ bath containing normal Ringer solution continuously aerated and maintained at 37 °C. The tissue was equilibrated under a load of 500 mg. The composition of the solution used was in mM litre^{-1}; NaCl 154; KCl 5·4; $CaCl_2$ 2·4; $NaHCO_3$ 6·0 and dextrose 11. In certain experiments the medium calcium content was reduced to 0·8 mM ($\frac{1}{3}$ normal).

Isotonic contractions were recorded on a smoked drum. After initial equilibration for 30 min, cumulative dose response curves were taken for various agonists, before and in the presence of naloxone (0·3 nM to 30 μM) as described by van Rossum & Van den Brink (1963). Each tissue was exposed to only one concentration of naloxone. The effective dose that produced 50% of the contraction (ED50) was calculated from a graph of log dose vs percent responses and it was expressed as negative logarithm of the molar concentration of the agonist. A change in ED50 was expressed as log shift. A significant decrease in ED50 was considered as potentiation. Statistical significance was tested using Student's *t*-test.

Drugs used: noradrenaline hydrochloride (NA) (Sigma) 5 nM to 50 μM; methoxamine hydrochloride (Burroughs Wellcome) 40 nM to 400 μM; acetylcholine chloride (ACh) (E. Merck) 50 nM to 5 mM, potassium chloride (KCl) 8, 16, 32, 80 mM and naloxone hydrochloride (Endo Lab) 0·3 nM to 30 μM.

RESULTS

Effect of naloxone (60 min) at various concentrations on the sensitivity of vas deferens to NA. Naloxone in the concentrations used, (0·3 nM to 30 μM) up to 120 min, did not itself produce any contraction of the tissue, nor at 0·3 nM and 30 nM did it produce significant changes in the ED50 of NA. But at 3 μM it significantly reduced the ED50 of NA while at 30 μM no further decrease in the ED50 of NA was observed (Table 1). The 3 μM concentration of naloxone produced the maximum potentiation.

Effect of naloxone (3 μM), *incubated at various time intervals, on NA responses.* Experiments with naloxone (3 μM) incubated at 30, 60 and 120 min reveal that naloxone produced potentiation in a time-dependent manner. There was a non significant trend towards potentiation in 30 min, but the effect was significant at 60 min and reached maximum; there was no further increase at 120 min.

Effect of naloxone (3 μM, 60 *min*) *on the response of vas deferens to various agonists.* Naloxone significantly potentiated the responses of the vas deferens to methoxamine, ACh and KCl by reducing their ED50 values (Table 2).

Influence of calcium ion on naloxone induced potentiation. When the tissue was incubated with low calcium Ringer solution, naloxone (3 μM, 60 min) did not produce any change in the sensitivity of the vas

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Table 1. Effect of naloxone at various concentrations (60 min) on the sensitivity of guinea-pig vas deferens to NA induced responses.

Treatment	Negative log molar ED50 ± s.e.m.	Log shift
Control	5·57 ± 0·06	—
Naloxone 0·3 nM	5·36 ± 0·11	−0·21
Naloxone 30 nM	5·67 ± 0·08	+0·10
Naloxone 3 μM	6·54 ± 0·12	+0·97*
Naloxone 30 μM	6·60 ± 0·10	+1·03*

Each value represents the average of at least six experiments.
+ indicates shift of ED50 to lower concn and − indicates shift of ED50 to higher concn
* Statistically significant with $P < 0·001$.

deferens to NA. So low calcium in the medium antagonized the potentiation produced by naloxone (Table 3).

In all the above experiments, the slope and the maxima of NA, methoxamine, ACh and KCl were reproducible.

Table 2. Effect of naloxone (3 μM) on the responses of guinea-pig vas deferens to various agonists.

Agonist	Negative log molar ED50 ± s.e.m. Control	Naloxone treated	Log shift
Methoxamine	4·44 ± 0·03	4·84 ± 0·05	+0·40**
Acetylcholine	4·87 ± 0·13	5·66 ± 0·14	+0·79**
Potassium chloride	1·65 ± 0·06	1·86 ± 0·06	+0·21**

Statistically significant from control value with * $P < 0·05$. ** $P < 0·001$.

DISCUSSION

In vascular smooth muscle, naloxone has been shown to inhibit contractions produced by KCl and NA. The present experiment shows that naloxone (3 μM) produces significant potentiation of the responses of guinea-pig vas deferens to NA and also it requires 60 min incubation to produce potentiation.

The possible mechanism of the potentiation of NA responses by naloxone was studied. NA responses can be effectively potentiated by inhibiting its re-uptake. Methoxamine, an α-receptor stimulant has very weak affinity for uptake sites when compared with NA (Burgen & Iversen 1965). So the effect of naloxone on methoxamine-induced responses was studied. Naloxone significantly potentiated the responses of methoxamine. This result is not in favour of the possibility that naloxone might be inhibiting the re-uptake of NA to potentiate its responses. Further experiments were conducted to study the specificity of naloxone action. Naloxone (3 μM) potentiated the responses of ACh and KCl by reducing their ED50 values. These observations led to the conclusion that naloxone produces a non-specific potentiation of the responses of the vas deferens to various agonists.

Table 3. Influence of calcium ion on naloxone-induced potentiation to NA in guinea-pig vas deferens.

Media	Negative log molar ED50 of NA ± s.e.m. Control	Naloxone treated	Log shift
Normal Ringer	5·57 ± 0·06	6·54 ± 0·12	+0·97*
Low Ca^{2+} Ringer	5·60 ± 0·10	5·71 ± 0·04	+0·11

* Statistically significant from control with $P < 0·001$

Calcium ion antagonizes most of the actions of morphine (Kakunaga et al 1966). Similarly, naloxone may also act through calcium to produce sensitivity changes. To test this possibility a solution containing low calcium was used and was found to antagonize naloxone-induced potentiation, indicating calcium dependency and suggesting that naloxone may favour calcium transport to produce potentiation.

In summary, naloxone produces non-specific potentiation on the guinea-pig isolated vas deferens in a dose-dependent and time-dependent manner. It might possibly produces its action by favouring calcium entry on the post synaptic site.

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