CHAPTER 6

SUMMARY AND CONCLUSIONS

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The present work studied the effects of opiate receptors and opioids on the response of isolated rat phrenic nerve diaphragm preparation to repeated electronic stimulation at a rate of 1/sec.

Morphine was used at three doses : 0.2mg%, 0.5mg% and 1.0mg%. The doses of naloxone used were 0.01mg%, 0.02mg% and 0.04mg%. The used drugs were added to the preparation bath at onset of experiment and the exposure time was 15min after which repeated stimulation were begun for 1/2h..

The study was carried out on 248 healthy albino rat of either sex. Their weight ranged from 140-160 gm. The isolated preparations were bathed in normal krebs solution in Group I and in four different Ca^{2+} concentrations in the krebs in Group II(A). Group II(B) and II(C) included the preparations bathed with different K^{+} and Na^{+} concentration in the krebs.

The results showed that :

(I) *In the first group : morphine at the dose 0.2mg% had no significant effect. Naloxone at the dose 0.02mg% decreased the indirect contraction of the diaphragm ($P < 0.05$) and the I/D% ($P < 0.001$) at the end of experiment. At the dose 0.04mg%, naloxone decreased the direct response ($P < 0.02$) after 15min. rest.*

(II) Both morphine and naloxone had no sig effect on the preparations bathed in Ca^{2+} free krebs, half normal Ca^{2+} in krebs and in double normal Ca^{2+} in krebs. However, morphine at the three used doses (0.2mg%, 0.5mg% and 1.0mg%) caused CNMB in some preparations bathed in 4- times normal Ca^{2+} in krebs before the end of experiments, and at the dose 0.2 mg% there was a decrease ($P < 0.02$) in the mean height of direct contraction at the end of experiment. However, the larger doses had no such effect. In contrast, naloxone only at the smaller dose (0.01mg%) had a stimulatory effect on both direct and indirect contraction ($P<0.02$) at the end of experiment.

(III) Morphine at the dose 0.5mg% stimulated the indirect contraction ($P < 0.005$) of the diaphragm bathing in K^+ free krebs at the end of exp., the I/D% was increased ($P < 0.02$). In contrast, morphine at the same dose inhibited the indirect response ($P < 0.05$) of the diaphragm bathed in half the K^+ of krebs solution after 15min. rest and at the end. Naloxone at the dose 0.02mg% had a stimulatory effect on the indirect contraction of the diaphragm bathed in 4 times normal K^+ in krebs solution.

(IV) The experiments done as regard the effects of morphine and naloxone in presence of Na^+ variations in the krebs solution were not sufficient to be studied.

In conclusion, the effect of both morphine and naloxone on the sequence of the isolated rat phrenic diaphragm preparation bathed in normal krebs

solution can not be concluded because of the restricted available doses. However, the variations in both Ca^{2+} and K^{+} concentrations in the krebs solution were good guides for understanding the mechanisms of actions of both morphine and naloxone on the preparations and also for providing regulatory effects of Ca^{2+} on opioid sites

Morphine and naloxone affect the rat phrenic diaphragm preparation by multiple mode of action :

(1) Naloxone (0.02mg%) may act on inhibitory opioid receptors at diaphragmatic endplate under normal condition (normal krebs solution).

(2) Morphine and naloxone may act on inhibitory-excitatory receptors on the rat diaphragm muscle enhanced by high extracellular Ca^{2+} concentration (4-times normal). Morphine has more affinity for the inhibitory receptors and naloxone has more affinity for the excitatory receptors.

(3) Morphine and naloxone may also act on inhibitory-excitatory receptors at rat diaphragmatic endplate giving inhibitory and excitatory effect, inhanced by the presence of high extracellular Ca^{2+} concentration (4 times normal), respectively.

(4) Morphine (0.5mg%) may facilitate the nicotinic site and/or may act as antiChE in absence of extracellular K^{+} concentration.

(5) Morphine (0.5mg%) may increase the inward K^{+} conductance at rat diaphragmatic endplate, in presence of low extracellular K^{+} concentration

(half the normal), causing inhibition of the indirect response of the rat diaphragm preparation.

(6) Naloxone at a dose 0.02mg% may have small excitatory effect on the phrenic nerve in presence of high extracellular K^+ concentration (4 times normal).

Yet, in the present study, opioid receptors subtypes were not be identified because the agonist (morphine) and antagonist (naloxone) were not tested together; they have limited specificity and the relative densities of different receptor subtypes in the present preparation are unknown.