ANTIACETYLCHOLINE ACTION OF IMIPRAMINE

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Imipramine, a potent antidepressive drug has been shown to antagonize the actions of a number of autopharmacological substances like histamine, 5-hydroxytryptamine, bradykinin, catecholamines and acetylcholine (1). Sigg and his coworkers (2, 3) observed a dual effect of imipramine on the various manifestations of the autonomic nervous system; a blocking action at higher dosage and a stimulant effect with low concentrations. The purpose of the present study was to investigate in more details the interactions between imipramine and acetylcholine at various sites, where acetylcholine is the neurotransmitter. This was done both at the muscarinic and nicotinic site.

MATERIALS AND METHODS

Studies on muscarinic sites

Smooth muscle preparations of several species of laboratory animals were studied. Tissues were mounted in an isolated organ bath in oxygenated Ringer Locke solution at 37±1°C unless otherwise mentioned.

i) Intestinal smooth muscle: Ileum of rabbit and guinea-pig, rat colon and taenia coli of guinea-pig were used. Imipramine was added to the bath in a concentration of \(1 \times 10^{-6}\) to \(1 \times 10^{-5}\) g/ml.

ii) Guinea-pig vas deferens and rat uterus: The latter being suspended in de Jalon solution at 22°C. Imipramine was used in the same range as before.

iii) Cardiac muscle: Isolated rabbit and frog hearts were employed for this purpose. The rabbit heart was perfused with Ringer Locke solution at 37±0.5°C, using Langendorff’s preparation. The frog heart was perfused with frog Ringer solution. Imipramine (10^-4 g/ml) was added to the perfusing fluid.

iv) Blood pressure: Mongrel dogs (6-14 kg), cats (2.5-4 kg) and albino rats (200-275 g) of either sex were employed for this purpose. Animals were anaesthetized with pentobarbitone sodium (30 mg/kg i.p.). Carotid blood pressure was recorded by a mercury manometer on a smoked kymograph paper and drugs were injected through the cannulated femoral vein in the case of dogs and cats, and jugular vein in rats. Imipramine was administered in a dose range of 0.5 to 5 mg/kg in all experiments.

Studies on nicotinic sites

A) Neuro-muscular junction

i) Sciatic-gastrocnemius preparation in the cat: Cats (3-4.5 kg) of either sex were anaesthetized with pentobarbitone sodium (30 mg/kg), and were maintained on positive
pressure artificial respiration. The contractions of the gastrocnemius muscle elicited by electrical stimulation of the sciatic nerve were recorded on a smoked kymograph paper. The sciatic nerve was stimulated submaximally by square wave pulses delivered by an electronic stimulator at a frequency of 15/min and of duration of 1 msec. Imipramine (0.5–5 mg/kg) was injected through the cannulated femoral vein. Carotid blood pressure was also recorded in these experiments.

ii) Rat phrenic nerve-diaphragm preparation: Phrenic nerve-diaphragm preparation was set up in an isolated organ bath of 30 ml volume in Tyrode solution (with double glucose) according to the method of Bulbring (4). The phrenic nerve was stimulated by submaximal square wave pulses of 10 msec duration at a frequency of 15/min from an electronic stimulator. Imipramine was added to the bath in a concentration of $1 \times 10^{-6}$ gm/ml.

iii) Frog rectus: Isolated frog rectus was put up in a 10 ml bath in oxygenated frog Ringer solution. Imipramine ($1 \times 10^{-6}$ to $1 \times 10^{-5}$ g/ml), was added in the bath.

B) Ganglionic site

The effect of imipramine on ganglionic transmission in the cat was studied by recording the contractions of the nictitating membrane following stimulation of the pre and post ganglionic fibres of the superior cervical ganglion.

RESULTS

Muscarinic actions

i) Intestinal smooth muscles

Imipramine ($10^{-6}$) completely inhibited the spontaneous contractions of the rabbit ileum, as well as blocked the action of acetylcholine (Fig. 1). However, the spontaneous contractions gradually reappeared after several washings and the tissue responded normally.

![Fig. 1. Effect of the same dose of acetylcholine on the isolated rabbit ileum before and after a single dose of imipramine.](image)
to acetylcholine. In the presence of imipramine, there was a parallel shift to the right of the dose response curve of acetylcholine.

Imipramine similarly blocked the response of ileum, taenia coli and vas deferens
of the guinea pig, and colon and uterus of the rat, to acetylcholine. In each case there was a parallel shift to the right of the acetylcholine dose response curve in the presence of imipramine. The representative dose response curves of acetylcholine on the guinea pig ileum has been shown in Fig. 2.

ii) Cardiac muscles

The presence of imipramine in the perfusion fluid reduced the amplitude of contraction of frog heart approximately by 25 percent and concurrently blocked the action of acetylcholine. In the isolated rabbit heart imipramine produced a marked reduction in amplitude of contraction (50%) and the effect of acetylcholine was similarly blocked.

iii) Blood pressure

Imipramine in the dosage used (0.5–5 mg/kg) invariably produced a fall in blood pressure in the dog, the cat and the rat. The fall in blood pressure was transient in lower dosage (0.5–3 mg/kg) and a prolonged effect was noted in higher dosage. Imipramine in above doses failed to modify the response of acetylcholine in blood pressure in any of the species studied. Typical response of imipramine and its effect on acetylcholine on rat blood pressure has been shown in Fig. 3.

Nicotinic sites

A) Neuromuscular junction

Imipramine did not modify the contraction of the gastrocnemius muscle of the cat in response to the electrical stimulation of the sciatic nerve.

In the presence of imipramine the amplitude of contractions of the rat diaphragm in response to the electrical stimulation of the phrenic nerve was gradually diminished leading to complete blockade. However, following repeated washings, there was recovery in 20 minute’s time. At the height of the blockade, neostigmine (1×10⁻⁶ g/ml) was unable to reverse it.

Likewise, in the isolated frog rectus preparation, imipramine (1×10⁻⁶ g/ml) reduced the response of the muscle to acetylcholine, and a complete blockade was observed in a concentration of 1×10⁻³ g/ml. There was a parallel shift of the dose response curve of acetylcholine to the right in presence of imipramine. However, imipramine was found to be ineffective when added to the bath after acetylcholine had produced a spasm.

B) Ganglionic transmission

Imipramine did not modify the contraction of nictitating membrane following stimulation of the cervical sympathetic chain of the cat. Though the contractions of nictitating membrane, was unaffected, the relaxation was incomplete.

DISCUSSION

In the present study, imipramine in higher doses blocked the action of acetylcholine in in vitro preparations. However, such blocking effect was not observed in various in vivo experiments. In the various smooth muscle preparations, there was a parallel shift of the log-dose response curve of acetylcholine in the presence of imipramine. Further, reappearance of the response of the tissues to acetylcholine following repeated washings
is suggestive of a reversible type of competitive antagonism.

Imipramine produced a depressor effect on the blood pressure in all the animal species studied. At a lower dose level the depressor action has been attributed to a central vaso-depressor effect (5). On the other hand, Sigg et al. (3) have attributed the hypotensive action of imipramine at a relatively higher dose level to a direct depressant action on the myocardium. In the present series of experiments also, a myocardial depressant action of imipramine was observed in the isolated heart of the frog and the rabbit. However, the antagonistic action of imipramine to acetylcholine was seen only in the isolated heart preparation, but not on the blood pressure response. This is in contrast to the findings of Osborne and Sigg (2) who reported that imipramine (6 mg/kg) could partially block the hypotensive action of acetylcholine.

Amongst the nicotinic sites, at the neuromuscular junction a blocking action of imipramine was evident in the frog rectus and in the rat phrenic nerve-diaphragm preparations. However, contrary to the observations of Sinha et al. (6) no blockade could be obtained on the cat sciatic gastrocnemius preparation. These observations, along with the fact that neostigmine failed to reverse the blocking effect in the rat phrenic nerve diaphragm preparation does not allow to draw any conclusion regarding the nature of antagonism.

Similarly at the autonomic ganglia intravenous administration of imipramine did not affect the acetylcholine action.

The fact that imipramine blocked the action of acetylcholine in all the isolated organ preparations irrespective of muscarinic or nicotinic sites, and its failure to do so in the intact preparations may point to the disparity between the effective concentrations of the test drug in basically two different types of experiments. Marked vasodepressor response of imipramine at 5 mg/kg precluded its use at further higher doses.

SUMMARY

Imipramine blocked the action of acetylcholine on smooth muscle preparations and on the heart. It could not block the muscarinic effect on the blood pressure response of the rat, the cat and the dog. Neuromuscular transmission was blocked in the rat phrenic nerve-diaphragm preparation and on frog rectus, but not in the sciatic-gastrocnemius preparation of the cat. Ganglionic transmission in the superior cervical ganglion of the cat was not blocked.

REFERENCES