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Interactions between acetylcholine, 5-hydroxytryptamine, nicotine and morphine on isolated rabbit atria

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1. The effects of 5-hydroxytryptamine (5-HT) and morphine on the responses to acetylcholine and nicotine of isolated rabbit atria were studied.

2. 5-Hydroxytryptamine (10 μg/ml.) and morphine (20 μg/ml.) blocked the negative chronotropic and inotropic actions of acetylcholine.

3. Nicotine (20 μg/ml.) produced stimulation of the atria, which was blocked by dichlorisoprenaline, morphine, 5-HT, bretylium and hemicholinium. Hemicholinium block was reversed by choline.

4. In reserpinized preparations, nicotine produced inhibition of atria and this action was also blocked by atropine, 5-HT and morphine. Inhibition induced by nicotine was potentiated by physostigmine.

5. 5-Hydroxytryptamine (20 μg/ml.) produced stimulation of atria. This was blocked by bretylium and reduced by hemicholinium. Hemicholinium block was reversed by choline.

6. It is concluded that 5-HT in low concentrations acts as a weak agonist at the cholinoceptive receptors and therefore blocks the action of acetylcholine. Furthermore, nicotine and larger doses of 5-HT have actions on ganglionic structures and liberate acetylcholine, which in turn releases catecholamines.

Although acetylcholine is known to have a negative inotropic action on isolated rabbit, atria, Hoffmann, Hoffmann, Middleton & Talesnik (1945) demonstrated that it produces stimulation in the presence of atropine and that this action is due to the release of catecholamines. Trendelenburg (1960) analysed the action of 5-hydroxytryptamine (5-HT) on isolated rabbit atria and postulated that 5-HT produced its effects through an action on neural elements in the atrial tissue. Jacob & Poite-Bevierre (1960) have shown that 5-HT has three successive phases of action on the isolated rabbit heart. The negative chronotropic and inotropic actions of 5-HT were shown to be blocked by atropine by these workers. In view of this, a study on the interactions of 5-HT and acetylcholine on isolated rabbit atria was undertaken. We have shown in the present investigation that 5-HT, in concentrations that fail to produce any discernible effects, influences the action of acetylcholine and nicotine on the atria.

Methods

The atria were dissected from the hearts of freshly killed rabbits (1.5–2.5 kg) and suspended at 30° C in oxygen saturated Locke's solution containing (per l.): 9.0 g NaCl, 0.42 g KCl, 0.24 g $CaCl_2$ 0.5 g $NaHCO_3$, and 2.0 g of dextrose. The pH was constant at about 8.0. The contractions of the atria were recorded on a smoked drum by a spring lever with minimal inertia. After addition of the drugs, the preparation was washed repeatedly and allowed to recover for at least 20 min before the next dose was added. When the effects of increasing concentrations of these drugs were studied, no control observations were made between successive doses of the same drug. Before studying the action of another drug, however, control responses to the test substance were always obtained.

Effects of varying concentrations of 5-HT and morphine on acetylcholine- or nicotine-induced responses of the atria were studied. Morphine or 5-HT was added to the fluid in reservoir so that the organ was continuously exposed to the drug. It was ascertained by an assay on rat uterus that 5-HT was stable in Locke solution over a period of 1 hr. In some experiments 5-HT was added to the bath directly as a single dose.

Some animals were pretreated with reserpine (2.5 mg/kg), administered by an intraperitoneal injection 48 and 24 hr before the experiment.

The following substances were used: 5-hydroxytryptamine creatine sulphate (5-HT), acetylcholine bromide, morphine sulphate, adrenaline HCl, nicotine, atropine sulphate, bretylium tosylate, dichlorisoprenaline (dichloroisoproterenol), hemicholinium HCl, triethylcholine, physostigmine salicylate, choline chloride and reserpine (Serpasil, CIBA). Drug concentrations are expressed in terms of μg/ml., referring to the final bath concentrations of the bases, with the exception of morphine, hemicholinium, triethylcholine, physostigmine, choline, atropine and bretylium, the concentrations of which are expressed in terms of the salts. Effects of drugs have been expressed as average percentage increase or decrease over the control and range of variation.

Results

Effect of 5-HT on the chronotropic and inotropic action of acetylcholine

Acetylcholine in concentrations of 0.1–1.6 μg/ml. of bath fluid produced a negative chronotropic and inotropic effect on the isolated rabbit atria. Acetylcholine was allowed to act for 2 min and was then washed out, whilst 5-HT (4.0 μg/ml.) was present in the bath for 20 min before addition of acetylcholine. This dose of 5-HT completely blocked the response to 0.1 and 0.2 μg/ml. of acetylcholine, while the response to 0.4 μg/ml. was reduced by 82% (78%–84%) and that to 0.8 μg/ml. and 1.6 μg/ml. of acetylcholine by 74% (69%–78%) and 56% (50%–66%), respectively. Washing of 5-HT from the bath restored the effect of acetylcholine. In the concentration used, 5-HT did not produce any action on the atria (Fig. 1). The blocking action of 5-HT was observed consistently in six experiments. This action of 5-HT was also seen in atria obtained from reserpinized animals. In three experiments on normal atria, acetylcholine (3.2 μg/ml.), in the presence of 5-HT (4.0 μg/ml.), produced a positive, rather than a negative, inotropic action [21.8% (19.1–24.6%)], although there was no change in the chronotropic action.

Effect of 5-HT and morphine on the responses to nicotine and adrenaline

Nicotine (1.0 μg/ml) produced positive chronotropic and inotropic effects [27.4% (23%–33%) and 93% (76%–140%), respectively; mean of three experiments]. This effect was seen in the absence of atropine and was thought to be due to the release of catecholamines. It was further noted that both the chronotropic and inotropic actions of nicotine were completely blocked by dichlorisoprenaline (1.0 μg/ml.). 5-HT, in concentrations of 10.0 μg/ml. and 20.0 μg/ml., produced a dose-dependent inhibition of the response to nicotine [33.2% in rate (30%–37%), and 65.7% in amplitude (63.3%–69%) with 10.0 μg/ml.; 67.0% in rate (64%–70.6%) and 78.6% in amplitude (75%–85.6%) with 20.0 μg/ml.; mean of three experiments]. Washing out of 5-HT restored the action of nicotine (Fig. 2). Morphine (20.0 μg/ml.) completely blocked the action of nicotine, but the block was reversible (Fig. 3). These results were obtained twice in each of the three experiments. Neither morphine nor 5-HT influenced the positive inotropic and chronotropic effects of adrenaline (0.05 μg/ml.).

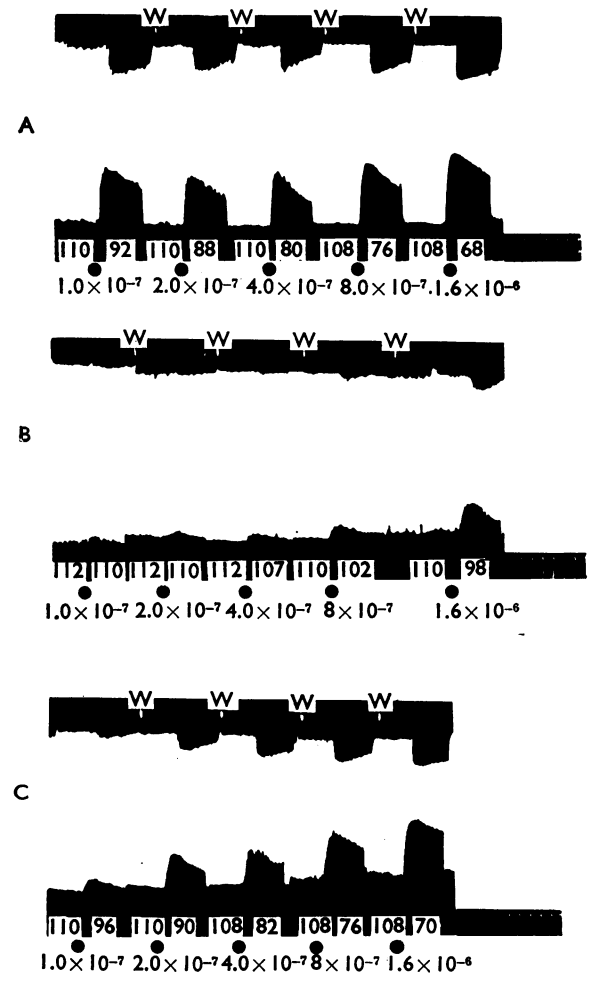

FIG. 1. Isolated rabbit atria. The effect of 5-HT in preventing inhibition of rate and amplitude in response to acetylcholine (0.1–1.6 μg/ml. bath). A, Control response to acetylcholine; B, responses to acetylcholine in the continuous presence of 5-HT (4.0 μg/ml.); C, responses to acetylcholine after washing out 5-HT. Numerals indicate heart rate per min. At the dots, acetylcholine added in concentration indicated. At W, repeated washing and drum stopped for 20 min. Time marker, 30 sec.

Effect of morphine on the responses to acetylcholine and KCl

Because the responses to nicotine were blocked by 5-HT and morphine and responses to acetylcholine were blocked by 5-HT, the effect of morphine on acetylcholine responses was studied. Morphine (5.0–20.0 μg/ml.) produced a progressive antagonism of acetylcholine (0.8 μg/ml.) responses [36% in rate (31.2%–39%), and 19.5% in amplitude (16%–22%) with 5.0 μg/ml.; 57% in rate (53%–61.2%) and 55.6% in amplitude (52.7%–59%) with 10.0 μg/ml.; 85.6% in rate (80%–89.6%) and 81% in amplitude (76.6%–86%) with 20.0 μg/ml.], without interfering with the inhibition produced by KCl (1.0 mg/ml.). The effect was noted twice in each of the three experiments.

Effect of bretylium on the responses of 5-HT and nicotine

In three experiments, the effect of bretylium (20.0 μg/ml.) on the responses to 5-HT (20.0 μg/ml.), and nicotine (1.0 μg/ml.) was studied. A single dose of 5-HT added to the bath or a single dose of nicotine was allowed to act for 3 min.

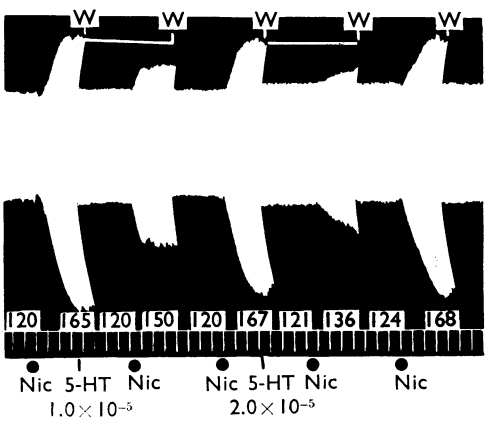

FIG. 2. Isolated rabbit atria. Effect of 5-HT on the increase in rate and amplitude caused by nicotine. At dots nicotine (Nic) (1.0 μg/ml.). Superior horizontal white lines indicate continuous presence of 5-HT in the bath in the concentration indicated. The tissue was exposed to 5-HT for 20 min (with drum stopped) before addition of the next dose of nicotine. At W, repeated washing and drum stopped for 20 min. Numerals indicate heart rate per min. Time marker, 30 sec.

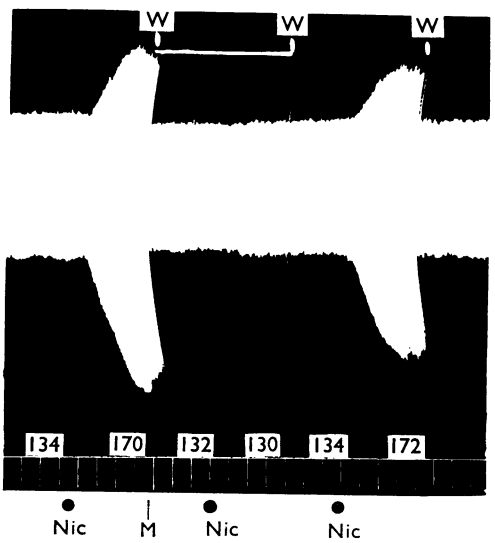

FIG. 3. Isolated rabbit atria. The effect of morphine in preventing the increase in rate and amplitude in response to nicotine. At dots, nicotine (Nic) (1.0 μg/ml.) was added. The tissue was exposed for 20 min to morphine (M) (20 μg/ml.) with drug stopped before addition of next dose of nicotine. Horizontal line indicates continuous presence of morphine. At W, repeated washing and drum stopped for 20 min. Numerals indicate heart rate per min. Time marker, 30 sec.

5-HT and nicotine in these concentrations caused a stimulation of the atria [18.7% in rate (16.8%–21%), and 82.6% in amplitude (72%–88%); and 25% in rate (23%–27%), and 78.3% in amplitude (76%–80%), respectively]. The stimulant action of these two drugs was blocked by bretylium (Fig. 4). In the presence of bretylium (20 min in the bath), 5-HT elicited negative chronotropic and inotropic actions [38.2% in rate (33.6%–42%), and 25% in amplitude (22.3%–29%)].

Effect of hemicholinium on the responses to 5-HT and nicotine

In three experiments the atria were exposed either to hemicholinium (100 μg/ml.) or triethylcholine (150 μg/ml.) for a period of 90 min. In these concentrations, neither drug had any action on the atria. The stimulant action of nicotine was completely blocked and that of 5-HT markedly reduced [67% inhibition in the inotropic (62%–73%), and complete block of chronotropic actions]. Exposure of atria to choline chloride (1.0 mg/ml.) for 90 min, after hemicholinium or triethylcholine, restored the actions of 5-HT and nicotine (Fig. 5).

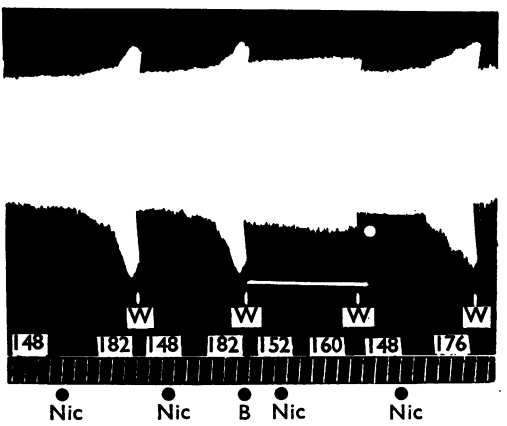

FIG. 4. Isolated rabbit atria. The effect of bretylium on the increase in rate and amplitude in response to nicotine. At dots, nicotine (Nic) (1.0 μg/ml.) and at B, bretylium (20 μg/ml.) was added for 20 min (with drum stopped) before the next dose of nicotine. Horizontal line indicates continuous presence of bretylium in the bath. At W, repeated washing and drum stopped for 20 min. Numerals indicate heart rate per min. Time marker, 30 sec.

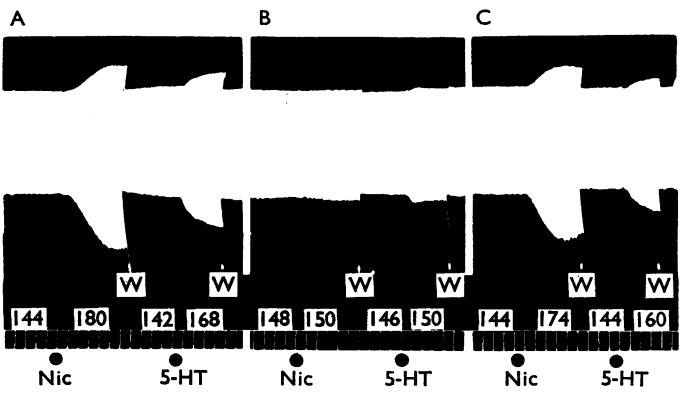

FIG. 5. Isolated rabbit atria. Effect of hemicholinium and choline on the increase in rate and amplitude in responses to 5-HT and nicotine. At dots, 5-HT (20.0 μg/ml.) and nicotine (Nic) (1.0 μg/ml.). A, Control response; B, responses after exposure to hemicholinium (100.0 μg/ml.) for 90 min and wash; C, responses after further exposure to choline (1.0 mg/ml.) for 90 min and wash. At W, repeated washing and drum stopped for 20 min. Numerals indicate heart rate per min. Time marker, 30 sec.

Effect of 5-HT, morphine, atropine and physostigmine on the response to nicotine of the atria obtained from reserpinized animals

In six experiments reserpinization reversed the action of nicotine on the atria. Thus, instead of stimulant action, nicotine in the same dose as used before now produced a negative chronotropic and inotropic action [20.1% in rate (18.4%–24%), and 29.9% in amplitude (26.2%–31.8%)], and this action was blocked by atropine but potentiated by physostigmine (Fig. 6). Furthermore, 5-HT and morphine antagonized this inhibitory effect of nicotine on the atria. The blocking effect of 5-HT and morphine was reversible, nicotine inhibition being restored after washing out the antagonists from the bath fluid.

Discussion

Because atrial stimulation by 5-HT (6.7 μg/ml.) was blocked by dichlorisoprenaline and was reduced in atria obtained from reserpinized animals, Trendelenburg (1960) postulated that 5-HT acts by releasing sympathin. When the atria were continuously exposed to varying doses of 5-HT (4–20 μg/ml.), the drug did not produce any stimulation; but addition of 20 μg/ml., as a single dose to the bath, elicited an action. 5-HT blocked effectively the negative chronotropic and inotropic actions of acetylcholine. In fact, in a few of our experiments, in the presence of certain concentrations of 5-HT, acetylcholine produced stimulation rather than inhibition.

The antagonism of acetylcholine by 5-HT could be explained in two ways: (i) 5-HT may release sympathin in the auricular tissue, thus antagonizing acetylcholine; (ii) there could be a pharmacological antagonism of acetylcholine by 5-HT at the receptor sites. The former possibility is ruled out by the finding that 5-HT, in low concentrations, failed to produce any positive chronotropic and inotropic action on the atria. Moreover, antagonism between acetylcholine and 5-HT was seen in the atria obtained from the reserpinized animals, indicating that the blocking action of 5-HT could not have been exerted through the release of sympathin. It is therefore necessary to accept the second possibility—that 5-HT and acetylcholine may be acting on the same receptor site.

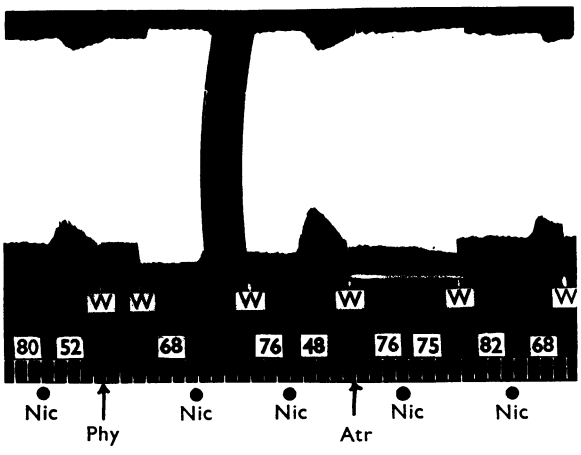

FIG. 6. Atria obtained from a rabbit pretreated with reserpine. Effect of physostigmine and atropine on the inhibition in rate and amplitude in response to nicotine. At dots, nicotine (Nic) (1.0 μg/ml.). At arrows, physostigmine (Phy) (2.0 μg/ml.) or atropine (Atr) (5.0 μg/ml.) allowed to act for 20 min (with drum stopped) before the next dose of nicotine. Horizontal line indicates continuous presence of atropine in the bath. At W, repeated washing and drum stopped for 20 min. Numerals indicate heart rate per min. Time marker, 30 sec.

Nicotine stimulated the atria, even in the absence of atropine. The stimulant action of nicotine was blocked by dichlorisoprenaline (Trendelenburg, 1960). In the atria obtained from the reserpinized animals, however, nicotine produced inhibition which was blocked by atropine and potentiated by physostigmine. In the absence of catecholamine stores, therefore, nicotine acts on the parasympathetic ganglia and produces cholinergic effects.

It is interesting to note that both the stimulant and inhibitory actions of nicotine are blocked by 5-HT. Because 5-HT was found to block the action of acetylcholine, it appears that nicotine may be producing its stimulant as well as inhibitory actions primarily through a release of acetylcholine. Such a mechanism of nicotine action has been reported by Leaders & Long (1962) in rabbit and cat atria. The stimulation is manifested only in the presence of stores of catecholamines.

A large dose of 5-HT (20 μg/ml.) as a single addition causes a stimulation of the atria, due to an action on neural elements, as with nicotine. This stimulant effect of 5-HT was blocked by choline-2-6-xylyl ether (TM-10) and cocaine (Trendelenburg, 1960). We have shown that the stimulant actions of both the drugs —5-HT and nicotine—are blocked by bretylium. Burn (1961) has proposed that bretylium may antagonize the release of sympathin by acetylcholine. There are no sympathetic ganglia in the heart, so it seems that acetylcholine, released at parasympathetic nerve terminals through stimulation of the ganglia by 5-HT and nicotine, may act on catecholamine stores to produce atrial stimulation and that this action of acetylcholine may be blocked by bretylium.

Hemicholinium inhibits the synthesis of acetylcholine (MacIntosh, Birks & Sastry, 1956). We have shown that continuous exposure of the atria to hemicholinium results in failure of response to nicotine and reduction of response to 5-HT. Harry (1963) showed that responses of guinea-pig ileum to 5-HT were reduced by hemicholinium, while those to nicotine were blocked at lower concentrations. Gulati & Panchal (personal communication) have demonstrated that, in atropinized rat ileum, the relaxant response to nicotine is blocked by hemicholinium, 3,6-di(3-diethylaminopropoxy) pyridazine di(methiodide) (Win 4981) and triethylcholine. It is therefore evident that the responses to nicotine, as well as to 5-HT, are mediated through a release of acetylcholine. This is further confirmed by our finding that choline restores the actions of nicotine and 5-HT, when blocked by hemicholinium. It is therefore not necessary to postulate an extraganglionic site for the positive chronotropic and inotropic action of nicotine, as suggested by Khan, Mantegazza & Piccinini (1965).

In our experiments, morphine blocked the responses of the atria to acetylcholine, as well as the stimulant and inhibitory responses to nicotine in normal atria and in atria obtained from reserpinized animals. It thus appears that morphine may act on the acetylcholine receptors in the atrial tissue directly.

Jacob and Poite-Bevierre (1960) have shown that atropine, which is a specific antagonist of acetylcholine, blocks the negative chronotropic and inotropic action of 5-HT. In the light of this finding, it appears that 5-HT may be acting as a weak agonist on acetylcholine receptors in the atria. The continuous presence of 5-HT in the bio-phase appears to block these receptors, thereby reducing the action of acetylcholine. The configuration of acetylcholine and tryptamine receptors appears to be similar.

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