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Absence of Nicotinic Receptors Mediating the Release of Norepinephrine from Adrenergic Neurons in the Rat Heart[1] (39960)

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Introduction. It appears well established that cholinergic receptors of the nicotinic type exist on some adrenergic nerve terminals (1-6), and that activation of these receptors results in a release of NE (4-8). Numerous drugs appear to have the ability to antagonize these responses (4, 5, 8). The physiological significance of these receptors remains obscure, although they have been postulated to mediate the cholinergic link in adrenergic transmission as proposed by Burn and Rand (9). The receptors are of great pharmacological interest since nicotine is the major ingredient in tobacco (10). In the course of experiments carried out to gain a better understanding of the action of nicotine on the release of NE from adrenergic nerve terminals experiments were carried out on the isolated perfused rat heart. The present studies demonstrate that unlike the rabbit, cat, dog, and guinea pig heart, nicotine does not induce a release of NE from the rat heart (5-8, 11, 12).

Methods. Male Sprague-Dawley rats weighing between 200 and 300 g were used for all experiments. Hearts were removed from rats under pentobarbital anesthesia and placed in an Anderson-Carver coronary perfusion apparatus (Metro Scientific Co.). The normal perfusion medium contained the following composition in millimoles per liter: NaCl, 119.8; KCl, 5.63; $CaCl_2$, 2.16; $MgCl_2$, 2.1; dextrose, 10.0; and $NaHCO_3$, 25.0. Ascorbic acid (10 mg/100 ml) and EDTA (10 mg/liter) were added to the perfusion medium to prevent oxidation of NE. The solution was bubbled with 95% O_2-5% CO_2; the temperature was 38 ± 1°, and pH 7.32 to 7.4. Hearts were perfused at a constant rate of 5.0 ± 0.25 ml/min. Following an initial equilibration period of 10 min, hearts were perfused with 1 ng/ml of *l*-[^{3}H]norepinephrine (specific activity 4.1 Ci/mmole) for 20 min to label the endogenous amine stores. The hearts were then switched to a norepinephrine-free medium and the efflux of norepinephrine was collected and analyzed for [^{3}H]norepinephrine. For guinea pig studies similar preparations were used. Hearts from male guinea pigs weighing between 200 and 400 g were removed under pentobarbital anesthesia and immediately connected to the coronary perfusion apparatus via the aorta. The perfusion medium was identical with that used for the rat hearts. All hearts were perfused at a constant flow of 6.0 ± 1.0 ml/min. Following a 10-min equilibration period, hearts were perfused with [1-^{3}H]NE (1 ng/ml) as described for the rat hearts. Parallel experiments on rat heart and guinea pig hearts were carried out simultaneously using the same preparation of [^{3}H]NE and drugs. After 10-20 min of perfusion with a norepinephrine-free medium, the rat or guinea pig hearts were challenged with nicotine (9.2×10^{-5} *M*), KCl (6×10^{-2} *M* KCl), or tyramine (2.3×10^{-3} *M*). It is assumed that the release of [^{3}H]norepinephrine from the heart reflects release from adrenergic nerve terminals because procedures which cause degeneration of the nerve terminals, such as 6-hydroxydopamine, are known to prevent drug-induced release of [^{3}H]NE from the heart (5).

Each releasing agent was infused into a side arm of the perfusion cannula at a constant rate for 1 min. Each heart received only one agonist. The perfusate effluents were continuously collected and [^{3}H]NE was analyzed by liquid scintillation spectrometry after alumina column chromatography (5). The concentration used for each drug was based on preliminary experiments carried out in the guinea pig heart, where complete dose-response curves were ob-

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tained. A concentration of each drug was chosen which was equivalent to an ED 70 in the guinea pig heart. Nicotine was studied in varying concentrations ranging from 10^{-8} to 10^{-2} *M*.

The uptake and accumulation of [^{3}H]NE was also determined in both the rat and guinea pig hearts. All hearts were removed from the animal and placed in the coronary perfusion apparatus as described above. The hearts were equilibrated with normal medium for 30 min and then switched to a medium containing [^{3}H]NE (1.0 ng/ml; sp act 4.1 Ci/mmole) for 10 min, followed by a 3-min drug-free medium. Ascorbic acid (100 μg/ml) and the disodium salt of EDTA (10 μg/ml) were added to the medium containing [^{3}H]NE to prevent oxidation. Hearts were removed at the end of the perfusion, weighed, and homogenized in 5% trichloroacetic acid. After filtration and adjustment of pH to 8.3–8.5, the catecholamines were adsorbed on alumina columns as described previously. Uptake and accumulation was expressed as disintegrations per minute of [^{3}H]NE per gram per 10 min.

The following drugs were utilized in the present study: *l*-[7-^{3}H]norepinephrine (New England Nuclear), nicotine hydrochloride (K and K Laboratories), nicotine sulfate (K and K Laboratories, Plamried, New York), tyramine hydrochloride (Sigma Chemical Co., St. Louis), dextroamphetamine sulfate (Smith Kline and French).

Results. Figure 1 shows a comparison of the effect of nicotine and potassium chloride on the release of [^{3}H]norepinephrine from the perfused guinea pig and rat heart. Both nicotine (9.2×10^{-5} *M*) and KCl (6×10^{-2} *M*) produced a marked increase in the release of [^{3}H]NE from the guinea pig heart. In contrast, this concentration of nicotine was completely without effect in altering the release of [^{3}H]NE from the rat heart, despite the fact that KCl produced a marked releasing effect. A complete range of nicotine concentrations going from 10^{-8} to 10^{-2} *M* failed to increase the efflux of [^{3}H]NE from the perfused rat heart.

The uptake of [^{3}H]NE, on the other hand, is quite similar into the hearts of both species (Fig. 2). Other drugs which are known to release NE were also studied in the rat heart. Figure 3 shows that tyramine, as anticipated, produced a marked increase in the release of [^{3}H]NE from the rat heart. There was a lack of effect, however, when acetylcholine (in the presence of atropine) was used as a nicotinic agonist.

No attempt was made to measure heart rate or contraction in the present study. However, it was readily apparent that both tyramine and amphetamine increased heart rate in the rat heart, while nicotine did not. In the guinea pig heart all three agonists

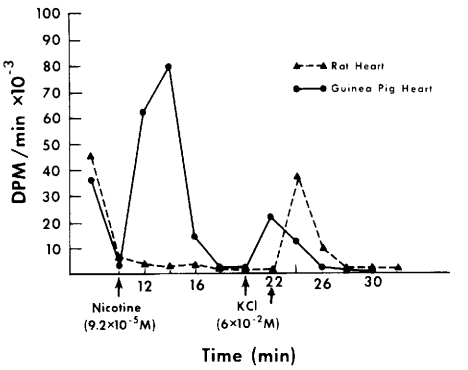

FIG. 1. The effect of nicotine and KCl on the release of [^{3}H]norepinephrine from the perfused rat or guinea pig heart. Data are plotted as [^{3}H]NE in disintegrations per minute $\times$ 10^{-3} (ordinate) vs time in minutes (abscissa). At the arrows either nicotine or KCl was administered as described in Methods. Each curve represents the mean data obtained from at least 10 hearts.

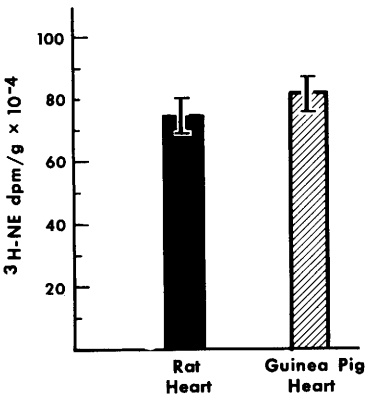

FIG. 2. The uptake of [^{3}H]NE into the perfused rat or guinea pig heart. Data are plotted as the mean uptake of [^{3}H]NE in disintegrations per minute per gram wet weight $\times$ 10^{-4} following perfusion with [^{3}H]NE for 10 min.

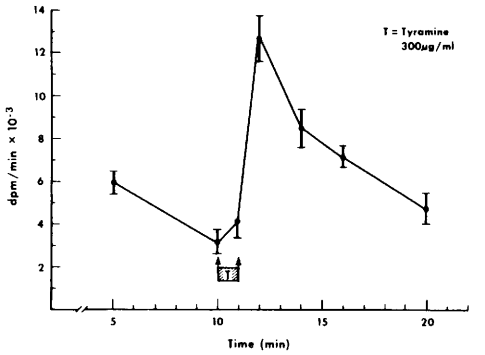

FIG. 3. The effect of tyramine on the release of [^{3}H]norepinephrine from the perfused rat heart. Data are plotted as disintegrations per minute $\times 10^{-3}$ (ordinate) vs time in minutes (abcissa). Tyramine was administered as described in Methods. Each point represents the mean ± SEM of 10 hearts.

produced a readily noticeable increase in heart rate. Previous studies in the guinea pig heart demonstrated that nicotine produced a dose-dependent increase in heart rate which was not blocked by atropine (5). A similar dose-dependent increase in the release of [^{3}H]NE was also seen which correlated very well with the increase in heart rate (5).

Discussion. It is well known that nicotine stimulates autonomic ganglia and the adrenal medulla (9, 13, 14). There is also convincing evidence that nicotine causes sympathomimetic effects by releasing norepinephrine from adrenergic nerve terminals (4, 5, 7, 15). The isolated perfused heart has been a popular model with which to study drug effects such as nicotine on adrenergic nerve terminals. It has previously been shown that nicotine releases NE from cat, rabbit, and guinea pig hearts (5–8, 11, 12). In the course of experiments to study the effect of nicotine in animals chronically exposed to nicotine in their drinking water, it was unexpectedly observed that nicotine did not release NE from the perfused rat heart. Presently a direct comparison of the effect of nicotine on the release of NE from rat and guinea pig hearts was made. Several preparations of nicotine, obtained from different commercial sources, were tried. Nicotine readily caused a release of NE from the guinea pig heart which is well known (5, 8, 12), but the same preparation of nicotine was without effect in causing a release of NE from the rat heart (Fig. 1). Differences in sensitivity between guinea pig and rat heart was ruled out by the fact that even at a concentration of 10^{-2} there was no release of NE. Prolonging the infusion of nicotine from 1 to 10 min was also without effect. In order to be sure that the hearts obtained from rats used in this study could take up and accumulate NE in the normal manner, uptake studies were performed. The uptake of NE into the rat heart was very similar to that of the guinea pig heart (Fig. 2).

In addition, following the uptake of NE into the rat heart, the amine could be released by three drugs well known for their ability to cause catecholamine release: potassium, tyramine, and amphetamine. However, the release of NE by nicotine could not be demonstrated. Despite the lack of a direct effect by nicotine on the release of norepinephrine from the rat heart, an increase in the myocardial turnover of norepinephrine in the rat has been observed following the chronic administration of nicotine (16). The present results would suggest, therefore, that the effect on turnover is mediated by a central mechanism rather than an effect at the level of the nerve terminal.

All experiments in the present study were conducted on rats of the Sprague–Dawley strain. It will be of great interest to determine if this lack of an effect on the nicotine-induced release of [^{3}H]NE from the perfused heart is similar in other rat strains. At present no information on this point is available.

The present results are also of great interest in view of the lack of a release of norepinephrine by nicotine or acetylcholine plus atropine from the isolated chicken heart (17). An inhibition of norepinephrine release mediated by muscarinic receptors has, however, been observed in the chicken heart. This latter action has been reported for a large number of adrenergically innervated tissues in several species (18). It has been argued that since nicotinic receptors mediating a release of norepinephrine were absent in the chicken heart, these receptors are not a ubiquitous property of all postganglionic sympathetic neurons (17). On the other hand, since muscarinic inhibitory re-

ceptors have been found in all tissues and species where they have been looked for, this argues in favor of a physiological role for muscarinic receptors and a lack of one for nicotinic excitatory receptors.

The results of the present study show that nicotinic excitatory receptors are absent from adrenergic nerve terminals in the rat heart. This present observation of a lack of nicotinic excitatory receptors on adrenergic neurons in a mammalian heart strongly supports the assertion of Engel and Löffelholz (17). It also suggests that the rat heart is a poor tissue with which to study the interaction of nicotine on adrenergic systems.

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