Acetylcholine Release from Rat Atria Can Be Regulated through an \( \alpha_1 \)-Adrenergic Receptor

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SUMMARY. Isolated superfused rat atria release \( ^3\text{H} \)acetylcholine when depolarized with 57 mm potassium. The depolarization-induced overflow of \( ^3\text{H} \)acetylcholine is markedly inhibited by micromolar concentrations of epinephrine and norepinephrine. The \( \alpha_1 \)-selective adrenergic agonist methoxamine also inhibits tritium overflow, but the \( \alpha_2 \)-selective adrenergic agonist clonidine and the \( \beta \)-adrenergic agonist isoproterenol do not. Prazosin, an selective \( \alpha_1 \)-adrenergic antagonist, blocks adrenergic inhibition of \( ^3\text{H} \)acetylcholine overflow with a \( K_i \) of approximately 0.4 nM. Yohimbine has approximately one-hundredth the potency of prazosin for blocking adrenergic inhibition of \( ^3\text{H} \)acetylcholine overflow. \( ^3\text{H} \)Norepinephrine overflow from isolated rat atria is also inhibited by norepinephrine, but this effect is antagonized by yohimbine and not by prazosin. We suggest that the release of acetylcholine from cardiac parasympathetic neurons can be regulated through an \( \alpha_1 \)-adrenergic receptor, and that this mechanism may underly, at least in part, the relative lack of effects of prazosin on heart rate. (Circ Res 56: 763–766, 1985)

ACETYLCHOLINE (ACh) is the neurotransmitter of the cardiac vagus and is the chemical signal by which parasympathetic activity modulates heart rate, impulse conduction, and contractility. The activity of the vagus nerve is known to be controlled centrally, but the possibility that there is local modulation of ACh release from cardiac parasympathetic neurons has not been adequately explored. We recently found that ACh overflow from parasympathetic neurons in isolated rat atria was inhibited by the sympathetic neurotransmitter norepinephrine and by the sympathetic hormone epinephrine (Wetzel and Brown, 1985). Further studies with selective agonists and antagonists are reported here. These studies demonstrate that the adrenergic receptor regulating ACh release does not have the \( \alpha_2 \)-adrenergic characteristics typical of presynaptic receptors but, instead, has the pharmacological properties of an \( \alpha_1 \)-adrenergic receptor (Starke et al., 1975; Wikberg, 1978; Hoffman and Lefkowitz, 1980; Langer, 1981).

Methods

Right atria from adult male Sprague-Dawley rats were incubated with \( ^3\text{H} \)choline to label acetylcholine stores, and \( ^3\text{H} \)acetylcholine release was quantified as previously described (Wetzel and Brown, 1985). Briefly, labeled atria were superfused with oxygenated Krebs-Henseleit buffer, and \( ^3\text{H} \)ACh release was stimulated by pulses of depolarization with buffer containing 57 mm K+. The superfusate was counted directly to measure total \( ^3\text{H} \)overflow, or \( ^3\text{H} \)ACh and \( ^3\text{H} \)choline were separated and quantified by high voltage electrophoresis (Potter and Murphy, 1967).

More than 95% of the \( ^3\text{H} \)overflow from atria stimulated by 57 mm K+ depolarization was \( ^3\text{H} \)ACh, whereas \( ^3\text{H} \)ACh comprised only 17% of the \( ^3\text{H} \)overflow from unstimulated atria (Wetzel and Brown, 1985).

RESULTS AND DISCUSSION

The naturally occurring catecholamines epinephrine (EPI) and norepinephrine (NE) inhibit the K+-stimulated overflow of \( ^3\text{H} \) by as much as 60%, decreasing the S1:S2 ratio to −0.3 (Table 1). The catecholamine concentrations that give half-maximal inhibition are \( 2 \times 10^{-5} \) and \( 6 \times 10^{-6} \) M for EPI and NE, respectively. In contrast, isoproterenol does not inhibit K+-stimulated \( ^3\text{H} \)overflow (Table 1). These data indicate that NE inhibits ACh overflow through activation of an \( \alpha \)-rather than a \( \beta \)-adrenergic receptor. This is supported by our previous observation that the effect of NE is blocked by yohimbine, an \( \alpha \)-adrenergic receptor antagonist, but not by the \( \beta \)-adrenergic receptor antagonist propranolol (Wetzel and Brown, 1985).

\( \alpha \)-Adrenergic receptors have been classified as \( \alpha_1 \),...
or α2, based on their location and pharmacological properties (Wikberg, 1978; Hoffman and Lefkowitz, 1980; Langer, 1981). Although EPI and NE interact with both α1 and α2 receptors at similar concentrations (Starke et al., 1975; Wikberg, 1978; Snively and Insel, 1982), other agonists show selectivity (i.e., greater relative potency) for one vs. the other receptor subtype. Methoxamine and clonidine are selective agonists at α1- and α2-adrenergic receptors, respectively (Wikberg, 1978; Hoffman and Lefkowitz, 1980; Langer, 1981; van Meel et al., 1981; Snively and Insel, 1982). We find that methoxamine significantly decreases K+stimulated [3H]ACh overflow from rat atria (S2:S1 = 0.50 ± 0.02) at a concentration of 10 μM. Methoxamine is a full agonist at α1-receptors in guinea pig aorta (Wikberg, 1978), but apparently does not have full activity in our preparation, since its maximal effect does not equal that of norepinephrine. The selective partial α2-agonist clonidine does not inhibit the K+stimulated overflow of [3H]ACh at 10 or 100 μM (Table 1).

Another means of distinguishing α1- and α2-adrenergic receptors is through the use of specific antagonists. We originally assumed that the receptor regulating ACh release would be an α2-adrenergic receptor, and therefore used yohimbine (10 μM) to block the effect of NE (Wetzel and Brown, 1985). Complete antagonist concentration-response curves (Fig. 1) reveal that prazosin, an antagonist with marked selectivity for the α1-adrenergic receptor subtype (Hoffman and Lefkowitz, 1980; Langer, 1981; Snively and Insel, 1982), is almost 100 times more potent than yohimbine. The Ki, calculated for prazosin inhibition of the effect of NE on [3H]overflow is approximately 0.4 nM. This is nearly identical to the Ka of the cardiac α1-adrenergic receptor for prazosin, as determined in radioligand-binding studies (Karliner et al., 1979; Williams et al., 1981). Thus, data with both agonists and antagonists suggest that the receptor mediating α-adrenergic inhibition of cardiac ACh release is an α1-adrenergic receptor.
methoxamine inhibit ACh release (Loffelholz, 1981, Loffelholz et al., 1984). Our studies provide direct evidence that catecholamines inhibit ACh overflow from the mammalian heart. Whether adrenergic inhibition occurs physiologically, in response to local sympathetic nerve stimulation or epinephrine release from the adrenal, remains to be determined. The latter possibility appears less likely, since plasma epinephrine concentrations are generally below those needed to inhibit release.

The observation that catecholamines inhibit $[^3]H\text{ACh}$ overflow from the rat heart is consistent with the finding that there is adrenergic regulation of ACh overflow from cholinergic neurons in the ileum (Paton and Vizi, 1969; Wikberg, 1978; Drew, 1978). In the ileum, however, it is an $\alpha_2$-adrenergic receptor that modulates transmitter release, whereas our data indicate that release of ACh in the heart is regulated through an $\alpha_1$-adrenergic receptor.

It is not possible to determine the precise location of the $\alpha_1$-adrenergic receptor responsible for modulation of $[^3]H\text{ACh}$ overflow. We have previously presented evidence that changes in $K^+$-stimulated $[^3]H\text{ACh}$ overflow are unlikely to be secondary to muscarinic or adrenergic effects on atrial muscle activity or to changes in impulse transmission through parasympathetic ganglia (Wetzel and Brown, 1985). We have also ruled out the possibility that the effect of NE on ACh release is secondary to $\alpha$-adrenergic receptor-mediated changes in prostaglandin forma-

tion (McDonough and Brown, in preparation). Nor-}

epinephrine could act by releasing other neurom-

modulators or transmitters from cardiac interneu-

rons. The most parsimonious explanation, however, is that norepinephrine acts directly on $\alpha_1$-adrenergic receptors located on the parasympathetic nerve endings.

The early notion that all postsynaptic adrenergic receptors are of the $\alpha_1$-subtype, whereas presynaptic adrenergic receptors are of the $\alpha_2$-subtype, has proven inadequate (McGrath, 1982). Indeed, several physiological studies suggest that $\alpha_1$-adrenergic receptors may be present presynaptically in the rat heart (Kobinger and Pichler, 1980; Docherty, 1984). The physiological importance of presynaptic $\alpha_1$-adrenergic receptors has been questioned because the primary regulation of norepinephrine release is clearly via $\alpha_2$-receptors (Docherty, 1984). The possibility that $\alpha_1$-adrenergic receptors play a significant physiological role by regulating the release of acetylcholine remains to be tested.

The parasympathetic nervous system predominates over the sympathetic nervous system in the control of heart rate (Levy, 1971). If the adrenergic receptor mechanism described here functions physiologically, it would provide a means for locally withdrawing this parasympathetic control by inhibiting ACh release when sympathetic tone is elevated. The ability of prazosin to block this adrenergic charge receptor mechanism, and thus dis inhibit ACh release, may explain why antihypertensive therapy with prazosin does not cause tachycardia (Cavero and Roach, 1980; Hoffman and Lefkowitz, 1980). We postulate that adrenergic inhibition of ACh release may involve a mechanism by which heart rate is normally increased. The cardiac parasympathetic neuron may therefore be a novel site for sympathetic-parasympathetic antagonism (Levy, 1971) that bears consideration in the design of adrenergic antagonists for cardiovascular drug therapy.

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