—SHORT COMMUNICATIONS

EFFECTS OF MUSCARINIC AGONISTS AND ANTAGONISTS
ON THE NEGATIVE CHRONOTROPIC RESPONSES TO
THE VAGUS NERVE STIMULATION

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Presynaptic receptors play an important role in regulating the release of neurotransmitters. Atropine has been shown to facilitate the release of acetylcholine (ACh) from brain. This was originally explained by a blockade of postsynaptic muscarinic receptors and subsequent disinhibition of cholinergic neurons through neuronal circuits in the brain (1). However, findings that atropine enhances the release of ACh from cortical slices in the presence of tetrodotoxin and high concentration of K^+ suggest a local regulation of the release of ACh (2). Furthermore, the release of ACh was inhibited by oxotremorine (3). Thus, a negative feedback mechanism in central nervous system (CNS), by which the release of ACh is regulated through depression of presynaptic muscarinic receptors by released ACh has been proposed. A possible regulatory mechanism of the release of ACh through presynaptic receptors in peripheral nervous system (PNS) also has been investigated. The failure of atropine to enhance the release of ACh from the superior cervical ganglion suggested the possible absence of the negative feedback mechanism in the PNS (4). However, recent studies have demonstrated the enhanced release of ACh from the myenteric plexus of guinea-pig ileum induced by atropine and its inhibition by oxotremorine indicating a participation of the presynaptic receptors in the regulation of the release of ACh (5, 6).

We studied the effects of methylatropine and methacholine on the negative chronotropic responses induced by the vagal nerve stimulation in rabbits. Male rabbits were anesthetized with urethane (1.75 g/kg, s.c.). The common carotid artery was cannulated to measure blood pressure using a Carrier Amplifier PR-3 transducer (NIHON KOHDEN) and an auricle vein was cannulated for infusion of drugs. Heart rate was obtained from the pressure pulse. Blood pressure and heart rate were recorded on a multipurpose recorder, Model RM-20 (NIHON KOHDEN). The vagus nerve was exposed at the cervical region and was stimulated with Ag-AgCl electrodes using an electronic stimulator at a subthreshold voltage (0.2–0.4 volt) with a duration of an impulse of 50 msec and at 5 Hz for a period of 10 sec. Changes of heart rate and blood pressure induced by the stimulation of the vagus nerve were measured at various intervals for a period of 30 min to obtain control responses. Fifteen min after control responses were obtained, methylatropine or methacholine was

infused i.v. at a rate of 0.5 ml/min in varying doses for 30 sec and responses to the vagal stimulation were re-examined for 30 min. Stimulation of the vagus nerve in the cervical region at the subthreshold voltage (0.2–0.4 volt) had no influence on heart rate and blood pressure. However, the vagus nerve stimulation at the same voltage following the infusion of methylatropine enhanced the negative chronotropic responses without significantly changing the blood pressure. The maximum reduction of heart rate (average 25% in 7 rabbits) was observed 3 min after the infusion of methylatropine (Fig. 1-A). The effect of methylatropine was reversible and was observed only when the vagus nerve was stimulated at a voltage ranging 0.2–0.4 volt. Methylatropine alone produced no measurable change in heart rate. The negative chronotropic responses caused by exogenously infused ACh were not affected by methylatropine (not shown). On the contrary, methacholine (1–3 $\mu\text{g/kg}$ i.v.), a muscarinic agonist, reversibly reduced the negative chronotropic responses to the vagal stimulation without affecting blood pressure. The maximum effect of 30% was observed at 3 min following the infusion of methacholine (Fig. 1-B). When nerve stimulation was not applied there was no effect on heart rate with these doses of methacholine.

Figure 2 shows the effect of various doses of methylatropine on the negative chronotropic responses. The results are expressed as a percent reduction of heart rate with various doses of methylatropine. The maximum effect was observed with 0.25 $\mu\text{g/kg}$ of methylatropine. Increasing the dose of methylatropine over 0.25 $\mu\text{g/kg}$ decreased the effect and blocked the negative chronotropic responses to the vagal stimulation. The principal finding of the

FIG. 1. Negative chronotropic responses to vagus nerve stimulation at various times following the infusion of methylatropine (A) and methacholine (B). The vagus nerve was stimulated at 0.2 volts (in A) and 0.4 volts (in B) with a duration of an impulse of 50 msec and at 5 Hz for a period of 10 sec. Average $\pm$ S.D. for observed values is shown ($n=3-7$). Significant differences from control: * $P<0.05$.

FIG. 2. Effect of various doses of methylatropine on negative chronotropic responses to vagus nerve stimulation. Changes in heart rate were measured 3 min following the infusion of methylatropine. Average $\pm$ S.D. for observed values is shown ($n=3-7$). Significant differences from control: * $P<0.05$.

present study is that the blockade of muscarinic receptors with methylatropine, a muscarinic antagonist which has little central action, caused a significant enhancement of the negative chronotropic response during the stimulation of the vagus nerve. On the other hand, methacholine, a muscarinic agonist which has selective effects on the cardiovascular system caused a significant inhibition of negative chronotropic responses during the stimulation of the vagus nerve.

These findings support the idea that muscarinic receptors are present in vagal nerve terminals and participate in feedback inhibition on release of ACh by the nerve stimulation. Furthermore, the affinities of agonists and antagonists to the pre- and post-synaptic muscarinic receptors may differ, as has already been reported in the case of α -adrenergic receptors (7, 8). Methylatropine and methacholine seem to have higher affinities for the presynaptic than the postsynaptic receptors in this system.

Alternative possibilities that these effects are mediated through ganglion or muscarinic receptors on the sympathetic nerve terminals (9) cannot be excluded.

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