Effect of \(\alpha\)-Ephedrine on Cholinergic Neurotransmission in the Isolated Guinea Pig Ileum

Ryuichi YAMAMOTO, Chikako NUKI, Hideyuki KOMIDORI\(^1\) and Koichiro TAKASAKI

Department of Pharmacology and \(^1\)1st Department of Internal Medicine, Miyazaki Medical College, 5200 Kiyotake, Miyazaki 889-16, Japan

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Abstract—In the isolated guinea pig ileum, the effects of \(\alpha\)-ephedrine on the twitch response to field stimulation were investigated in the presence of propranolol. Ephedrine and clonidine inhibited the twitch response but not the contraction to exogenous acetylcholine. The inhibitory effect of clonidine was significantly diminished by yohimbine pretreatment. However, the inhibitory effect of ephedrine was not influenced by yohimbine, sulpiride or 6-hydroxydopamine (6-OHDA) pretreatment. Most of this action of ephedrine appeared to be cholinergic prejunctional in nature, but unrelated to activation of prejunctional \(\alpha_2\)-adrenoceptors and dopamine sensitive receptors on the cholinergic nerves in this preparation.

Electrical field stimulation of the isolated guinea pig ileum causes acetylcholine release which is reduced by \(\alpha\)-adrenoceptor agonists (1, 2) or dopamine (3–5). The inhibition of acetylcholine release by \(\alpha\)-adrenoceptor agonists is mediated by prejunctional \(\alpha_2\)-adrenoceptors on the cholinergic nerves in this preparation (6, 7). Bauer (7) suggested that in the isolated guinea pig ileum, ephedrine could be a suitable \(\alpha_2\)-adrenoceptor agonist. However, he based his reasoning on only the fact that the effects of ephedrine mimic the results obtained by other \(\alpha\)-adrenoceptor agonists such as noradrenaline and adrenaline.

In this paper, we examined whether ephedrine is able to inhibit the twitch response to field stimulation through the activation of presynaptic \(\alpha_2\)-adrenoceptors on the cholinergic nerves in isolated guinea pig ileum. In our preliminary experiments, \(\alpha\)-ephedrine is a potent antagonist on twitch response to field stimulation, while \(d\)- and \(dl\)-ephedrine are weak ones. Thus, \(\alpha\)-ephedrine was used in this experiment.

Male or female guinea pigs, each weighing 300–400 g, were used. The animals that had been fasted for 12 hr before the experiments were killed by cervical dislocation. The terminal portion of the ileum was removed, and the last 20 cm discarded. After carefully washing out the luminal contents, the segments of ileum (2–2.5-cm long) were selected from the terminal portion and suspended under an initial tension of 1 g in Krebs’ solution at 37°C in a 30 ml organ bath. The composition of the Krebs’ solution was: 120 mM NaCl, 5 mM KCl, 2.4 mM CaCl\(_2\), 1.2 mM MgSO\(_4\)-7H\(_2\)O, 25 mM NaHCO\(_3\), 1.2 mM KH\(_2\)PO\(_4\), and 11 mM glucose; in addition, the solution contained propranolol (3 \(\mu\)M) to block \(\beta\)-adrenoceptors, and it was bubbled continuously by a mixture of 5% CO\(_2\) and 95% O\(_2\). The preparations were stimulated electrically with supramaximal square wave pulses, 1 msec in duration, delivered at a frequency of 0.1 Hz from an electronic stimulator connected to two platinum electrodes placed on either side of the ileum. Contractions of the longitudinal muscle were recorded isometrically, with a strain gauge, and displayed on a chart recorder. Atropine (3 \(\mu\)M) abolished the twitch responses to field stimulation, confirming that they were produced by cholinergic nerve stimulation (n=3). When the twitch height had become constant (after about 30 min), ephedrine, tyramine or
clonidine was added to the bathing fluid in a cumulative-concentration schedule. The interval between the exposure to increasing concentrations of these drugs was adjusted to allow the effect of each dose to develop fully. On each preparation, only one concentration-response curve was obtained. There was no significant difference between their cumulative and non-cumulative concentration-response curves. Yohimbine (0.1 nM) or sulpiride (100 nM) was added to the bathing fluid at 10 min before ephedrine or clonidine. For the chemical sympathectomy of the ileum, the animal was pretreated with 6-OHDA at doses of 50 and 100 mg/kg which was dissolved in 0.9% saline containing 0.1% ascorbic acid and administered i.p. on day 1 and day 2, and the preparation was made on day 3, 24 hr after the second administration. Additional experiments were performed in which the effect of ephedrine or clonidine on contractile responses to exogenous acetylcholine (0.001–1 μM) was studied in unstimulated preparations.

The concentration-response curves were analyzed by two-way analysis of variance (ANOVA). In all cases, the statistical significance was assigned at P<0.05.

Ephedrine (3–300 μM) and clonidine (1–100 nM) inhibited the twitch response to field stimulation in a dose-dependent manner (Figs. 1a, 2), but not the contraction to exogenous acetylcholine. Tyramine (3–300 μM), however, failed to inhibit the twitch response to field stimulation at any concentration used in this experiment (Fig. 1b). The inhibitory effect of clonidine on the twitch response to field stimulation (pD₂=8.16±0.07) was significantly diminished by yohimbine pretreatment (Fig. 2b). From this observation, it is likely that the inhibitory effect of clonidine on the twitch response to field stimulation is due to decreased acetylcholine release through prejunctional α₂-adrenoceptors on cholinergic nerves. It is well-known that ephedrine and tyramine exert most of their action on α-adrenoceptors through release of endogenous catecholamines from noradrenergic nerves. The result obtained by tyramine suggested that the inhibitory effect of ephedrine on the twitch response to field stimulation was unexplained by the catecholamines releasing action of this drug. There is further evidence to support this suggestion. The inhibitory effect of ephedrine was unaffected by chemical sympathectomy induced by pretreatment with 6-OHDA. Ephedrine required high concentrations for inhibition of the twitch response to field stimulation (pD₂=4.41±0.10). However, it would seem unlikely that the inhibitory effect of ephedrine on the twitch response to field stimulation is due to nonspecific action of this drug, because the contractile response curve to exogenous acetylcholine was not significantly influenced by ephedrine at any concentration used in this experiment. It is also unlikely that the inhibitory effect of ephedrine is due to blockade of the cholinergic postjunctional muscarinic receptors.

These results indicated that most of the inhibitory effect of ephedrine on the twitch response to field stimulation is cholinergic prejunctional in nature. However, the inhibitory effect of ephedrine on the twitch response to field stimulation was not significantly influenced by pretreatment with yohimbine or sulpiride (Fig. 2a). These results suggested that the inhibitory effect of ephedrine is not induced by direct and/or indirect activation of prejunctional α₂-adre-
noceptors and dopamine sensitive receptors on cholinergic nerves. Recently, Bond et al. (8) suggested that norepinephrine-induced inhibition of the guinea pig ileum twitch response is mediated by two distinct sites: one is the classical prejunctional \( \alpha_2 \)-adrenoceptor, whereas the other is a site seemingly unrelated to the \( \alpha \)- and \( \beta \)-subtypes. Thus, the inhibitory effect of ephedrine on twitch response to field stimulation seemed related to such unknown prejunctional receptors on cholinergic nerves.

On the other hand, high concentrations of tyramine (such as 100 and 300 \( \mu \)M) induced smooth muscle contraction (Fig. 1b). Bauer (7) reported that in the guinea pig ileum, there are at least two \( \alpha \)-adrenoceptors (inhibitory prejunctional-\( \alpha_2 \), stimulatory post-junctional-\( \alpha_1 \)) and an inhibitory post-junctional \( \beta \)-adrenoceptor. Furthermore, tyr-
Amine has been suggested to activate α-adrenoceptors directly (9-11). Thus, the tyramine-induced smooth muscle contraction may be due to direct stimulant action on postjunctional α₂-adrenoceptors.

Further study is necessary to determine the inhibitory effect of ephedrine on exocytotic acetylcholine release from the cholinergic nerves in the isolated guinea pig ileum.

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