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Influence of α_2-Agonists and Antagonists on Acetylcholine Release in the Circular Muscle of Rat Jejunum

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The aim of the present experiments was to measure the release of acetylcholine directly from the circular muscle strips of rat jejunum and to investigate α_2-adrenoceptor agonists and antagonists involvement in this release. The selective α_2-adrenoceptor agonist UK-14,304 (1 μM) significantly inhibited electrically evoked release of [^{3}H]-acetylcholine. This effect was prevented by the α_2-adrenoceptor antagonist rauwolscine (2 μM), suggesting that presynaptic α_2-adrenoceptors are present on cholinergic neurons of the rat jejunum. Both basal and electrically-evoked release of acetylcholine were not affected significantly in the presence of rauwolscine or guanethidine, suggesting that in the rat jejunum sympathetic nerves do not modulate acetylcholine release via the inhibitory α_2-adrenoceptors.

Key words: Acetylcholine release, α_2-adrenoceptors, ileum, jejunum.

Introduction

The small intestine belongs to the affected area in human postoperative ileus [4], and rodent models are used to understand the pathogenesis of the disturbed motility in postoperative ileus. In order to study the influence of operative procedures on gastrointestinal motility, it is important to know its exact regulation under normal conditions. The majority of noradrenergic fibres, innervating the gastrointestinal tract, end on intrinsic enteric neurons rather than on smooth muscle cells, and exert their effect by modulating the activity of intrinsic neurons. In general, noradrenergic activity inhibits non-sphincteric gastrointestinal smooth muscle by inhibition of acetylcholine release from intrinsic excitatory cholinergic motor neurons via α_2-adrenoceptors [7]. This has also been shown at the level of the stomach: α_2- or α_2-like adrenoceptors have been shown to be present on postganglionic cholinergic neurons in the dog gastric fundus [6]. The aim of this study, therefore, was to measure acetylcholine release directly and to investigate the influence of α_2-agonists and antagonists on the electrically induced acetylcholine release in the rat jejunum.

Materials and Methods

Male Wistar rats (250-350 g) were used in the experiments. All animals had free access to water and commercially available rat chow. Rats were sacrificed by stunning and subsequent decapitation. The circular muscle preparations of jejunum were prepared as described before [8]. The release of acetylcholine from jejunum smooth muscle strips was measured directly according to the method described before [5].

Results

The results from 13 experiments with control animals are summarized in Fig. 1. Ninety minutes after starting the superfusion, the spontaneous efflux of [^{3}H]-acetylcholine from the strips, preloaded with [^{3}H]-choline-chloride, reached a steady state. The strips were stimulated twice (S_1 and S_2) for 2 min by application of electrical field stimulation (5 Hz, 40 V, 1 ms). The first stimulation started at 5th sample, while the second stimulation started at 27th sample (Fig. 1). The electrical stimulation (S_1 and S_2) significantly increased the release of [^{3}H]-acetylcholine from the smooth muscle strips (Fig. 1). Pretreatment with 3 μM tetrodotoxin prevented the electrically-stimulated release of [^{3}H]-acetylcholine (Fig. 2), suggesting that [^{3}H]-acetylcholine was released from neuronal elements of the intestine. The selective α_2-adrenoceptor agonist UK-14,304 (1 μM), added 7 min before the second stimulation period, did not alter the basal efflux of [^{3}H]-acetylcholine (data not shown). However, UK-14,304 significantly decreased the amount of [^{3}H]-acetylcholine released upon electrical stimulation (Fig. 3). The α_2-adrenoceptor antagonist rauwolscine (2 μM) prevented the inhibition of the electrically evoked release of [^{3}H]-acetylcholine by UK-14,304 (Fig. 3). Since UK-14,304 was dissolved in 100 %

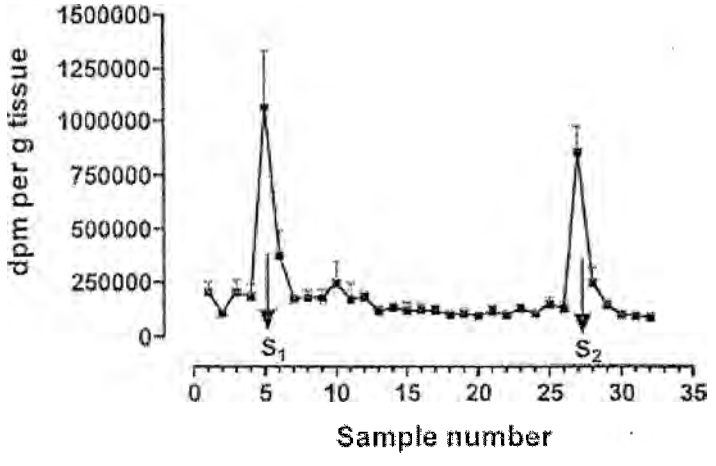

Fig. 1. Spontaneous and electrically-induced release of [^{3}H]-acetylcholine in circular smooth muscle strips from rat jejunum. The strips were stimulated twice (S_1 and S_2) for 2 min by application of electrical field stimulation (5 Hz, 40 V, 1 ms). The first stimulation started at 5th sample (13th min), while the second stimulation started at 27th sample (79th min). Results are expressed as disintegrations per minute (dpm), and normalized to the gram weight of the tissue. The means ± SEM of 13 experiments in the control rats are presented

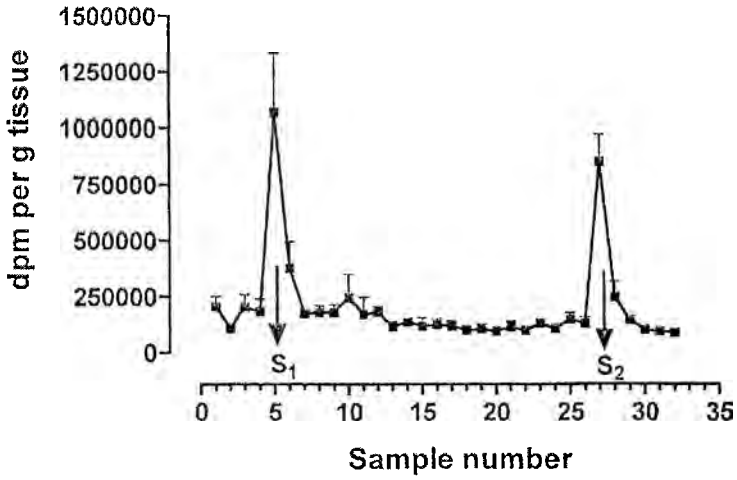

Fig. 2. Effect of tetrodotoxin (TTX) on [^{3}H]-acetylcholine release from circular smooth muscle strips of rat jejunum. The strips were stimulated twice (S_1 and S_2) for 2 min by application of electrical field stimulation (5 Hz, 40 V, 1 ms). Tetrodotoxin (3 μM) was added 10 min (24th sample) before S_2. Results are expressed as disintegrations per minute (dpm), and normalized to the gram weight of the tissue. The means ± SEM of 5 experiments are presented

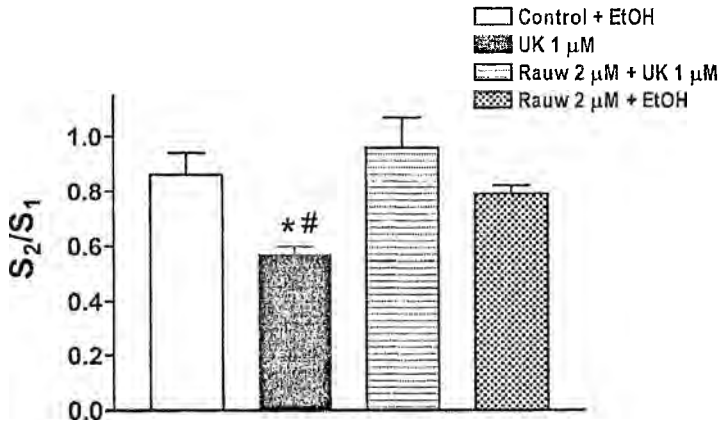

Fig. 3. Effects of UK-14,304 (UK), rauwolscine (rauw), and UK-14,304 in the presence of rauwolscine, on the [^{3}H]-acetylcholine release from circular smooth muscle strips of rat jejunum. The strips were stimulated twice (S_1 and S_2) for 2 min by application of electrical field stimulation (5 Hz, 40 V, 1 ms). The electrically evoked [^{3}H]-acetylcholine efflux is expressed as S_2/S_1 ratio for each muscle strip. Rauwolscine (2 μM, dissolved in distilled water) was added 37 min before S_2, while ethanol (4 μl) or UK-14,304 (1 μM, dissolved in 100 % warm ethanol) were added 7 min before S_2. Each column represents the mean ± SEM (n = 6), $^*P < 0.05$ versus control group; $^\#P < 0.01$ versus rauwolscine + UK-14,304 group

warm (37 °C) ethanol, the same amount of ethanol (4 ml) was added 7 min before the second stimulation to both control and rauwolscine-treated strips.

Discussion

Several investigators have reported that presynaptic inhibitory α_2-adrenoceptors are presented on cholinergic nerves in various gastrointestinal tissues and in other tissues [2]. In this study, the α_2-adrenoceptor agonist UK-14,304 significantly decreased the electrically-induced efflux of [^{3}H]-acetylcholine in the rat jejunum. This inhibitory action of UK-14,304 was completely antagonized by rauwolscine, a selective α_2-adrenoceptor antagonist, indicating that cholinergic nerves of the rat jejunum are also endowed with α_2-adrenoceptors, causing inhibition of transmitter acetylcholine release. To study whether endogenous noradrenaline is able to inhibit acetylcholine release via the presynaptic α_2-adrenoceptors on the cholinergic neurons, we performed experiments also in the absence of guanethidine. Both basal and electrically-evoked release were not lower compared to the release in the presence of guanethidine, suggesting that in rat jejunum sympathetic nerves do not modulate the release of acetylcholine via the inhibitory α_2-adrenoceptors within the experimental conditions and/or that electrical field stimulation does not lead to the release of noradrenaline. This was confirmed when rauwolscine was added between S_1 and S_2: it was without effect on the electrically-evoked release of [^{3}H]-acetylcholine. Also in the guinea-pig ileum and rat trachea [1, 3] no evidence to suggest that endogenous noradrenaline influences acetylcholine release was obtained. In conclusion, our results suggest: i) The presence of presynaptic muscarinic and α_2-adrenoceptors on the cholinergic neurons in the rat jejunum; ii) Endogenous noradrenaline is not inhibiting acetylcholine release via the presynaptic α_2-adrenoceptors.

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