Autoinhibition of Acetylcholine Output from Cholinergic Nerves through Activation of Muscarinic Receptors in the Guinea-Pig Ileum and Circular Muscle Preparations

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Autoinhibition of Acetylcholine Output from Cholinergic Nerves through Activation of Muscarinic Receptors in the Guinea-Pig Ileum and Circular Muscle Preparations

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SUMMARY

The significance of muscarinic receptors in the feedback autoinhibition of acetylcholine output evoked by distension and exposure to external excess potassium was investigated in the isolated guinea-pig whole intestine and circular muscle preparation. From the effect of tetrodotoxin, it was concluded that about 40% of the output from the intestine is due to propagated activity in the plexeus. The output of acetylcholine in the whole intestine in response to circumferential distension was enhanced by atropine (5—500nM), but depressed by muscarinic receptor agonist, oxotremorine (20 μM). However, external excess potassium-induced acetylcholine output was only insignificantly increased by atropine. Acetylcholine output by circular muscle preparation was unaffected by atropine or oxotremorine. The results indicate that acetylcholine output from interneuronal cholinergic nerves is regulated by presynaptic inhibitory muscarinic receptors in the guinea-pig ileum.

INTRODUCTION

It has been demonstrated that acetylcholine will be released spontaneously in central and peripheral cholinergic nervous system[~5] if incubated in the presence of cholinesterase inhibitors. However, the rate of spontaneous acetylcholine output fell progressively, as the collection period was increased[6]. The possibility arises that acetylcholine accumulation in the synaptic cleft is itself inhibiting the further output of the neurotransmitter. It is generally accepted that a large dose of muscarinic receptor antagonists inhibit gastro-intestinal motility, but a low dose enhanced it[7,8]. Evidence that the release of acetylcholine from neurones in the myenteric plexeus of the small intestine may be regulated by noradrenergic inhibition, in this case mediated by presynaptic alpha-adrenergic receptors, has been reported from several laboratories[9~12].

The present experiments were done to examine the possibility that the dual-mode model proposed for autoinhibition of noradrenergic nerves may apply also to the feedback autoinhibition of acetylcholine release from cholinergic nerves. The test applied for this purpose was comparison of the effects of muscarinic agonists and antagonists on the release of acetylcholine evoked by indirect depolarization of the nerve terminals with circumferential distension, with those on the release by direct depolarization of the terminals with external excess potassium.

MATERIALS AND METHODS

Guinea-pigs of either sex weighing 250 to 400g, were stunned by a blow on the head and bled out instantaneously from the carotid artery to death. A length of small intestine was quickly removed,

with abuot the 10cm proximal to the ileocecal sphincter being discarded. The segments of the ileum, 4 to 5cm in length were mounted in a 1.5ml organ bath containing Tyrode solution of the following composition (mM) : NaCl, 136. 9 ; KCl, 2. 7 ; $CaCl_2$, 1.8 ; $MgCl_2$, 0.5 ; $NaHCO_3$, 11.9 ; NaH_2PO_4, 0.4 ; glucose, 10.0. The bathing medium was kept at 37℃, aerated with 95% O_2 and 5% CO_2 and pH was adjusted to 7.2—7.4. In most cases the medium contained eserine at 50μM that completely protects acetylcholine from hydrolysis by the acetylcholine esterase in the tissue.

When it was desired to stimulate the segments with distension of the intestinal wall, the segments were distended circumferentially by inserting a glass rod of 7mm diameter. In experiments to study the effect of external excess potassium, changes in the tonicity of the medium were minimized by adding sodium chloride at concentrations equimolar with potassium chloride level used during stimulations.

All the samples collected were kept on ice until the biological assay. At the end of the experiments, the amount of release of acetylcholine resulting from each stimulation was calculated by subtraction of the resting output from the total output during stimulation. The procedure for biological assay of acetylcholine was described in detail in a previous paper[14].

The following drugs were used : acetylcholine chloride (Wako), physostigmine salicyate (Sigma), oxotremorine (Aldrich), atropine sulfate (Sigma). A certain amount of the concentrated drug solution was added to the bathing solution.

The results are expressed as mean ± S. E. of the mean. Statistical evaluations of the results were carried out with Student's t test.

RESULT

Comparison of the acetylcholine content and output of the whole intestine with that of circular muscle preparations

For the analysis of acetylcholine release mechanisms, the resting acetylcholine output from whole ileum and from circular muscle preparations of ileum isolated from the same animal were compared. The resting outputs occurring with both preparations were as follows : 62.5±4.9ng/g wet weight tissue/min (n =12) from whole ileum and 9.1±2.3ng/g wet weight tissue/min (n =12)from circular muscle preparations (Fig. 1). These outputs are normally steady for the first 40 min of an experiment, but sometimes tend to decline after this period by approximately 30%. The resting output from the whole ileum is roughly 7 times as high as that from circular preparations. The acetylcholine contents of preparations of small intestine and of circular muscle strip isolated from the same animals were compared. The content of the whole ileum, 7.8±1.0μg/g wet weight tissue (n =9), was about 5 times as high as that of circular muscle preparations, 1.5±0.4μg/g wet weight tissue (n =9).

Acetylcholine output evoked by stimulation with distension and with external excess potassium

Table 1 shows that tetrodotoxin in a concentration of 3.5μM reduced the resting output by about 40%. Although distension of the whole ileum induced increased the output of acetylcholine, its response to distension stimulation was abolished by omitting calcium from the bathing medium, and was thus presumably exocytotic in nature. Tetrodotoxin depressed by it more than 90%, indicating that it was not due to direct depolarization of varicosities.

To study the releasing response to direct depolarization of varicosities external excess potassium was used as releasing stimulus. With both preparations of gut and circular muscle strip there is a considerable increase in acetylcholine output as a result of external excess potassium stimulation (Table 2). The releasing response to excess potassium of both preparation was unaffected by tetrodotoxin, but abolished exposure of the preparations to calcium-free medium. Therefore, this response was presumably exclusively due to direct depolarization of varicosities.

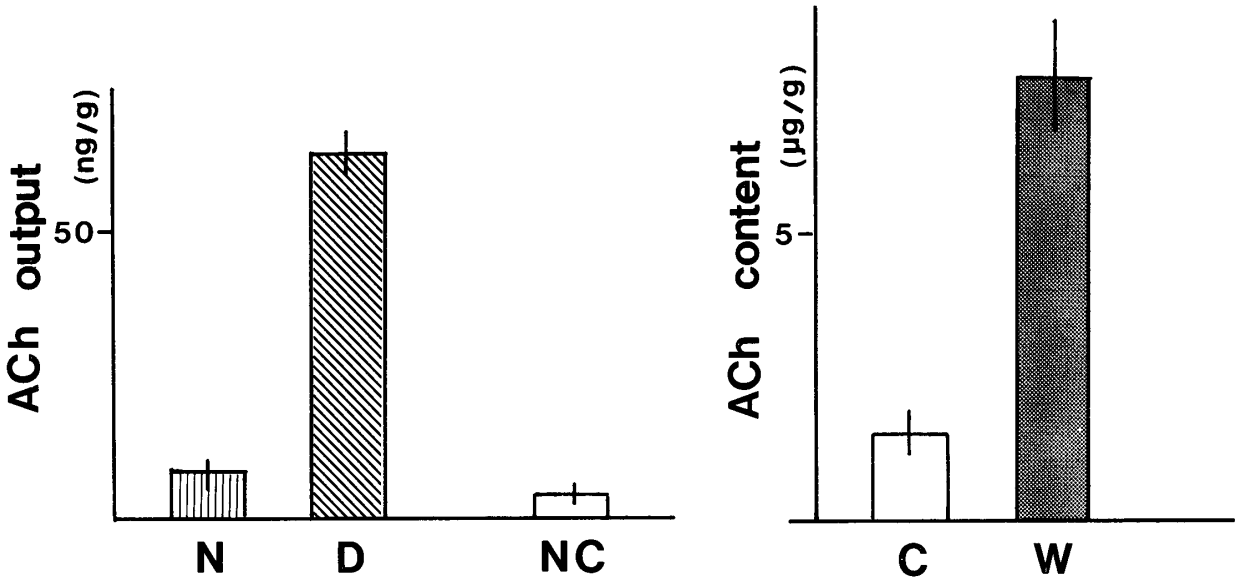

Fig. 1. Acetylcholine (ACh) outout and content. (A) the acetylcholine output evoked by circumferential distension of the isolated guinea-pig ileum. The experiments were carried out for 10min in the presence of 50μM physostigmine. (B) the acetylcholine content of both preparations of whole ileum and circular muscle isolated from the same animals. N,undistended intestine. D,circumferential distended intestine. W,whole intestine. C,circular muscle preparation. The preparation kept at 0℃ for 30min. (NC).

Effect of exogenous muscarinic agonist

As shown in Figure 2, addition of oxotremorine (20μM) decreased the output of acetylcholine evoked by distension of the whole ileum by about 40% ($n=5$) of control value, but only insignificantly depressed it to excess potassium medium. Also, the resting output of both preparations was not significantly depressed by its muscarinic receptor agonist ($p>0.05$). This action of an exogenous muscarinic receptor agonist demonstrates the presence, presumably on the cholinergic terminals, of functional presynaptic muscarinic receptors, activation of which depresses acetylcholine output.

Effect of atropine

The acetylcholine output evoked by distension of the whole ileum was enhanced in a concentration-dependent manner by atropine (5—500nM). The maximal enhancement was estimated to be 160% of control value (Fig. 3). In other experiments it was demonstrated that the releasing response to stimulation with external excess potassium (54mM) was not significantly enhanced by atropine (50—500nM), the response being 117.5±9.8% ($n=7$) of control value.

Table 1. Effect of calcium-lack and tetrodotoxin (TTX) on the output of acetylcholine evoked by circumferential distension of the intestinal wall. Experiments were carried out for 15 min in the presence of 50μM physostigmine. Calcium-free solution was prepared by omitting calcium chloride from the bathing solution. N,undistended intestine. D,circumferential distended intestine. Each value represents the mean ± S. E. of the mean in 4 to 12 experiments.

	Acetylcholine output (ng/g wet weight tissue/min)
N	65.3± 9.6(n=12)
N+TTX (3.5μM)	60.5±10.2(n= 4)
N+Ca^{++}-free medium	8.6± 2.4(n= 5)
D	117.7±14.4(n=12)
D+TTX (3.5μM)	68.5± 2.9(n= 5)
D+Ca^{++}-free medium	10.4± 3.5(n= 5)

DISCUSSION

In the present study, acetylcholine output evoked by distension of the intestinal wall was inhibited by oxotremorine and enhanced by atropine, strongly suggesting that the acetylcholine releasing mechanism at cholinergic nerve terminals is under the control of endogenous acetylcholine (a feedback autoinhibition). If the enhancement of acetylcholine output after the treatment with muscarinic receptor blocking agent is due to blockade of a cholinergic autoinhibitory process, then it can be assumed that in the presence of cholinesterase inhibitor, sufficient amounts of endogenous acetylcholine are present in the vicinity of presynaptic muscarinic receptors. The question has arisen whether the acetylcholine released under physiological conditions in the absence of cholinesterase inhibitors can reach inhibitory muscarinic receptors before being hydrolysed. In the guinea-pig ileum, it seems possible that small amounts of acetylcholine are spontaneously released during the resting state of the nerve, and that this acetylcholine inhibits the neuronal release of acetylcholine during resting and active states.

It has previously been shown that the distension stimuli applied to the myenteric plexues are transmitted aborally along Auerbach's plexues networks to neighboring ganglion and then evoke acetylcholine release in the anal region. Also, myenteric neurones appeared to receive cholinergic inputs from mechano-sensitive neurones throught nicotinic receptors

Table 2. Effect of calcium-lack and tetrodotoxin (TTX) on the output of acetylcholine evoked by exposure to external excess potassium. Solution with increased $[K^+]_o$ were obtained by replacing Na^+ by an equivalant amount of K^+. N,undistended intestine. Experiments were carried out for 15 min in the presence of 50μM physostigmine. Each value represents the mean ± S. E. of the mean in 4 to 10 experimentns

	Acetylcholine output (ng/g wet weight tissue/min)
N	60.7± 8.4(n=10)
27mM K^+medium	81.3±10.2(n= 4)
54mM K^+medium	102.1±11.7(n= 6)
54mM K^+medium +TTX (3.5μm)	98.3± 9.9(n= 6)
54mM K^++Ca^{++}-free medium	63.5±10.4(n= 5)

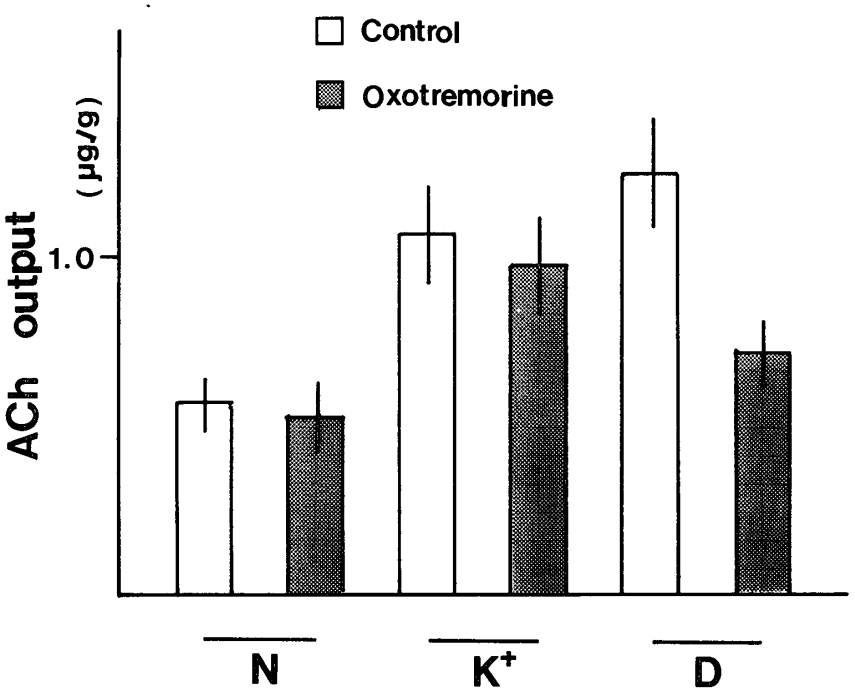

Fig. 2. Effect of oxotremorine on the output of acetylcholine (ACh) evoked by circumferential distension and by exposure to external excess potassium. The experiments were carried out for 15min in the presence of 50μM physostigmine. N,undistended intestine. K,+ exposure to external excess potassium (54mM). D,circumferential distended intestine.

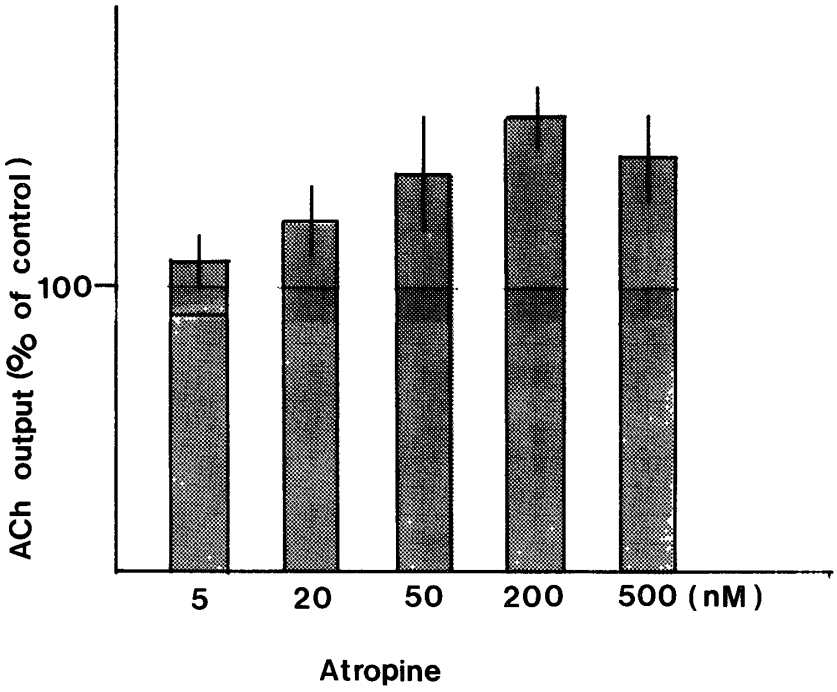

Fig. 3. Concentration-response relationship of the effect of atropine on the output of acetylcholine (ACh) evoked by circumferential distension. The experiments were carried out for 15min in the presence of 50μM physostigmine. Relative magnitude is represented as a percentage of the acetylcholine output evoked by circumferential distension before application of atropine.

since the release of acetylcholine is inhibited by ganglionic blocking agents[15~17]. When the results with whole intestine and circular muscle preparation are compared, it is found that the plexues attached to the whole intestine accounts for about one fifth of the acetylcholine content of the circular muscle preparation and for around one seventh of the resting output. With three possible types of extrinsic, interneuronal and pre-effector cholinergic fibres, acetylcholine release could occur either spontaneously or due to action potentials. In this study, it seems likely that in the resting state about 40% of the resting acetylcholine output depended on propagated activity. A similar partition of miniature junction potential discharge into tetrodotoxin-sensitive and tetrodotoxin-insensitive has been made at the neuromuscular junction on nerve-striated muscle preparations. It is known that tetrodotoxin abolishes propagated activity in the nerve fibres but retains spontaneous quantal release of acetylcholine at nerve terminations[18,19]. It is clear that the acetylcholine output consists of two main fractions, one associated with propagated activity and sensitive to ganglionic blocking agents and tetrodotoxin, the other not. The augmented output evoked by external excess potassium, unlike part of the resting output, is resistant to tetrodotoxin. Presumably, nerve conduction is impaired by the exposure to high potassium concentration.

It would be expected that the magnitude of the feedback inhibition of acetylcholine output depends not only on the concentration of acetylcholine in the vicinity of prejunctional muscarinic receptor, but also on the time for which this acetylcholine was kept in contact with the small intestine. It is possible that acetylcholine by depolarizing the nerve terminals inhibits calcium entry normally evoked by distension stimuli and thereby reduces the transmitter release.

Endogenous catecholamine or prostaglandins also regulate the release of acetylcholine, both in the resting and active states of the nerve terminal[20,21]. It is also known that low concentrations of histamine act on prejunctional terminals of the cholinergic nerve to enhance the release of acetylcholine through activation of neuronal H_1-receptors[22]. Thus, neural and hormonal factors including acetylcholine itself influence the extent of acetylcholine release at neuro-effector junction in the gut with a resultant decrease or enhancement of cholinergic neurotransmission.

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モルモット回腸と輪走筋標本における アセチルコリン遊離の前シナプス抑制

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モルモットの回腸標本と輪走筋標本を用いて，伸展刺激や高濃度K^+により遊離されるアセチルコリンのムスカリン受容体を介するフィードバック抑制機構が調べられた。回腸の内壁伸展刺激により誘発されるアセチルコリン遊離はアトロピンにより促進されるが，オキソトレモリンにより抑制される。しかし，高濃度K^+により遊離されるアセチルコリンはアトロピンで影響を受けない。輪走筋標本において遊離するアセチルコリンもムスカリ受容体アゴニストやアンダゴニストで変化しない。以上の結果は，モルモット回腸においてコリン作動性神経から遊離されるアセチルコリンは前シナプス性ムスカリン受容体により制御されていることを示している。

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