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The muscarinic receptor subtype in mouse pancreatic B-cells

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Isolated mouse islets were used to identify the muscarinic receptor subtype present in pancreatic B-cells. We thus compared the inhibitory potencies of atropine (non-specific), of pirenzepine (specific for M_1 receptors) and of compound AF-DX 116 (specific for cardiac M_2 receptors) on acetylcholine-induced insulin release, $^{86}Rb^+$ efflux and $^{45}Ca^{2+}$ efflux. The three antagonists inhibited all effects of acetylcholine, but EC_{50} values were markedly different: atropine = 1.5–5 nM, pirenzepine = 0.6–1.7 μM and AF-DX 116 = 1.7–11 μM. The results did not suggest that the various effects of ACh could result from the activation of different subtypes of receptors. It is concluded that muscarinic receptors of pancreatic B-cells belong to an M_2 subtype distinct from the cardiac M_2 receptors.

Acetylcholine; Muscarinic receptor; Pirenzepine; Insulin release; Ion flux; (Pancreatic islet)

1. INTRODUCTION

Stimulation of muscarinic receptors (mAChR) triggers a broad spectrum of biochemical and biophysical events [1]. The diversity of these cellular responses may be linked to the existence of distinct subtypes of receptors, activating different transduction pathways. The first evidence for the heterogeneity of mAChR was based on the differential affinity and inhibitory potency displayed by the antagonist pirenzepine (PZ) in different tissues [2,3]. An M_1 subtype, with high affinity for PZ, was identified in the central nervous system and sympathetic ganglia, whereas an M_2 subtype, with low affinity for PZ, was found in peripheral effector organs. However, a new antagonist, AF-DX 116, with a high affinity for M_2 receptors of the heart and a low affinity for M_2 receptors of exocrine glands, has led to further subdivision [4–6]. This pharmacological concept of mAChR heterogeneity was amply supported by recent molecular cloning studies, which identified no less than four distinct mAChR, with very large homologies in man, pig and rat [7–11]. Expression of these receptor subtypes varies between tissues, but the links between each subtype and a defined pathway of signal transduction are only recently beginning to be unravelled [11].

It has long been known that ACh modulates insulin secretion by activating mAChR of pancreatic B-cells [12,13]. Several mechanisms including acceleration of phosphoinositide hydrolysis, mobilization of intracellular calcium and changes in several ionic permeabilities of the plasma membrane are involved, but their precise links and relative importance remain under debate [13–18]. The present study was an attempt to identify the mAChR subtype(s) mediating some of these effects of ACh in B-cells. We thus compared the ability of atropine, PZ and AF-DX 116 to antagonize ACh-induced insulin release, $^{86}Rb^+$ efflux and intracellular Ca^{2+} mobilization in isolated mouse islets.

2. MATERIALS AND METHODS

Islets were isolated after collagenase digestion of the pancreas of fed female NMRI mice. They were then incubated for 90 min at 37°C in a control medium, or in a medium supplemented with a trace amount of $^{86}RbCl$ or $^{45}CaCl_2$. For studies of insulin release, batches of 3 islets were subsequently incubated for

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60 min in 1 ml of medium containing the test substances. At the end of the incubation, a portion of the medium was appropriately diluted for measurement of immunoreactive insulin, with rat insulin as standard. The techniques and the dynamic system of perifusion used to monitor the efflux of $^{86}Rb^{+}$ (tracer for K^{+}) or $^{45}Ca^{2+}$ from preloaded islets have been described in detail [17,19]. During the experiments of $^{45}Ca^{2+}$ efflux, a portion of each effluent fraction was also saved for measurement of insulin.

The medium used was a bicarbonate-buffered solution [14] containing 10 mM glucose in all experiments. Acetylcholine chloride was obtained from Sigma (St. Louis, USA), atropine sulfate from Merck (Darmstadt, FRG), and the radioisotopes from the Radiochemical Center (Amersham, England). Pirenzepine and AF-DX 116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-*b*][1,4]benzodiazepine-6-one) were kindly provided by Thomae GmbH (Biberach, FRG).

Depending on the phenomenon studied, each antagonist was tested at 3–5 concentrations in the presence of one concentration of ACh. The control response to ACh alone and the test responses in the presence of all concentrations of one antagonist were obtained in each experiment of incubation aimed at measuring insulin release. For technical reasons, however, efflux experiments could not always compare the control response to ACh alone and the test responses in the presence of all concentrations of one antagonist with the same preparation of islets. After subtraction of basal responses (without ACh), test responses in the presence of an antagonist were expressed as a percentage of the control response with ACh alone. To calculate the potency of the antagonists, the average concentration-response curves were first linearized by the least square method after probit transformation of the percentages [20]. The concentration producing 50% inhibition of ACh effects (EC_{50}) was obtained from the regression plot.

3. RESULTS

The control effects of ACh are shown in fig.1. At the concentration of 1 μM, ACh produced a ten-fold increase in insulin release by islets incubated in a medium containing 10 mM glucose and 2.5 mM Ca^{2+}. The same concentration of ACh also caused a marked and sustained acceleration of $^{86}Rb^{+}$ efflux from islets perifused with a Ca^{2+}-free medium (fig.1B). At the higher concentration of 100 μM, ACh triggered a prominent but transient peak of $^{45}Ca^{2+}$ efflux from islets perifused with a Ca^{2+}-free medium. This peak, which therefore reflects intracellular Ca^{2+} mobilization, was accompanied by a short-lived release of insulin (fig.1D). The strong inhibitory effect of 10 nM atropine attests to the muscarinic nature of all these effects of ACh.

The 3 muscarinic antagonists caused a dose-dependent inhibition of ACh-induced insulin release, $^{86}Rb^{+}$ efflux and $^{45}Ca^{2+}$ efflux (fig.2). However, their potencies were widely different. Comparison of EC_{50} values shows that PZ was about 240–400 times less potent than atropine, and that AF-DX 116 was 3–7 times less potent than PZ (table 1). EC_{50} values could not be reliably estimated for the inhibition of the peak of insulin release triggered by ACh in the absence of Ca^{2+} (fig.1D) because it was already markedly decreased

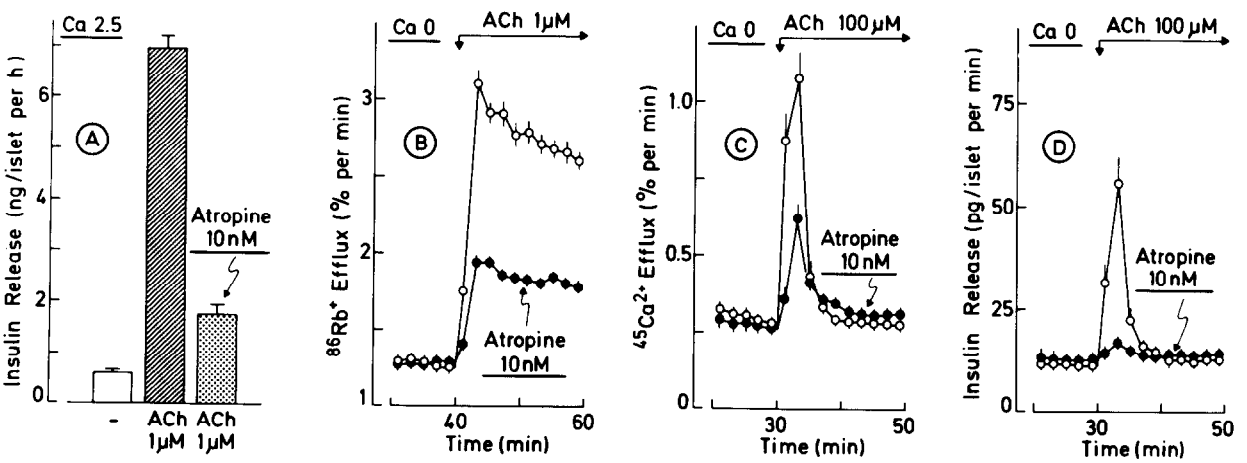

Fig.1. Inhibition by 10 nM atropine of various effects of ACh in mouse islets incubated in the presence of 2.5 mM Ca^{2+} (A) or perifused in the absence of Ca^{2+} (B–D). The concentration of glucose was 10 mM in all experiments. In A, ACh, with or without atropine, was present during the whole incubation. In B–D, ACh was added as indicated by the arrows whereas atropine, when tested, was present during the whole experiment. Values are means ± SE for 26–74 batches of islets (A), and for 4 test (•) and 8 control (○) experiments of efflux (B–D). Panel D illustrates the changes in insulin release measured during the experiments of $^{45}Ca^{2+}$ efflux (C).

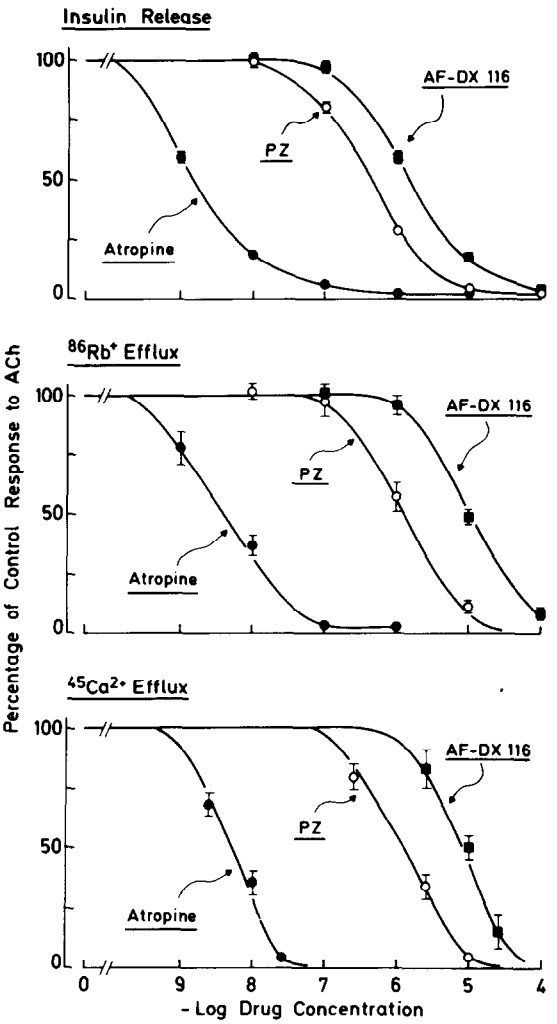

Fig.2. Concentration dependence of the inhibition of ACh effects by atropine, PZ and AF-DX 116. Results are expressed as a percentage of the control response to ACh alone, above basal values without ACh. These control responses are shown in fig.1. For efflux experiments, basal values correspond to the average rate just before addition of ACh (36–40 min or 26–30 min); test values correspond to the average rates between 42 and 60 min ($^{86}Rb^{+}$ efflux) or between 32 and 36 min ($^{45}Ca^{2+}$ efflux). Values are means ± SE (when larger than the symbol) for 24–26 batches of islets from 4 separate experiments (insulin release) or 4 experiments of efflux.

by the lowest concentrations of the antagonists. Compared to control values, this peak amounted to only 18 ± 3% with 2.5 nM atropine, 31 ± 4% with 250 nM PZ and 52 ± 7% with 2.5 μM AF-DX 116.

Table 1

EC_{50} values for atropine, PZ and AF-DX 116 antagonism of ACh effects in mouse islets

	Atropine (nM)	PZ (μM)	AF-DX 116 (μM)
Insulin release	1.45	0.55	1.65
$^{86}Rb^{+}$ efflux	4.20	1.70	10.7
$^{45}Ca^{2+}$ efflux	5.00	1.20	8.15

Values were calculated from the data of fig.2, as described in section 2

At the highest concentrations tested, atropine (10 μM), PZ (100 μM) and AF-DX 116 (100 μM) had no effect on insulin release, $^{86}Rb^{+}$ efflux or $^{45}Ca^{2+}$ efflux in the absence of ACh (not shown).

4. DISCUSSION

The nature of the mAChR subtype present in pancreatic B-cells was addressed in only one previous study [21]. From experiments using only two concentrations of antagonists, it was suggested that about 300 times more PZ than atropine is needed to inhibit carbachol-induced insulin release from the perfused rat pancreas. This estimation agrees with our finding of an EC_{50} ratio of 380. A similar low sensitivity to PZ (EC_{50} in the μM range) was also observed here when its effects on $^{86}Rb^{+}$ and $^{45}Ca^{2+}$ efflux were tested. One may thus reasonably conclude that B-cells do not possess mAChR of the M_1 subtype. Since the cardioselective M_2 antagonist AF-DX 116 was even less potent than PZ, it appears that mAChR of B-cells, like those of exocrine glands [5,6], differ from the cardiac M_2 subtype.

There are no direct arguments to identify these glandular M_2 mAChR of B-cells as M_3 or M_4 receptors. However, the M_3 subtype appears to be expressed mainly in tissues where ACh causes inhibition of adenylate cyclase [11], and this is not the case in B-cells [13]. On the other hand, the M_4 subtype is expressed in the exocrine pancreas [11]. In the latter tissue [22], as in B-cells [13–18], muscarinic agonists cause phosphoinositide hydrolysis, Ca^{2+} mobilization, changes in several ionic fluxes and secretion. The glandular M_2 subtype could turn out to be an M_4 mAChR.

Several, but not necessarily all effects brought

about by ACh in B-cells are interconnected [17,18]. Slight differences in the potencies of the antagonists on ACh-induced insulin release and on $^{86}Rb^{+}$ or $^{45}Ca^{2+}$ efflux were noted. However, these differences are too small to suggest involvement of distinct mAChR. They are more likely to be due to distinct experimental conditions (e.g. incubations in the presence of Ca^{2+} and perifusions in the absence of Ca^{2+}) in particular since the EC_{50} values of the non-specific antagonist atropine were similarly affected. Even the high sensitivity of the peak of insulin release induced by ACh in the absence of Ca^{2+} is easily explained. This peak is due to Ca^{2+} mobilization (peak of $^{45}Ca^{2+}$) by inositol trisphosphate. However, concentration-dependence studies have shown that the relationship between insulin release and the magnitude of Ca^{2+} mobilization is non-linear [18]. A small decrease in mobilization, as that produced by the lowest concentration of antagonists, is sufficient to cause a marked reduction in release. The presence of only one type of mAChR in B-cells would not necessarily imply that ACh triggers one single primary response, which, in turn, activates secondary responses in parallel or in sequence. Indeed, evidence has been obtained that a single mAChR subtype is able to mediate several biochemical responses [23].

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