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Characterization of Acetylcholine-induced Insulin Secretion in the Isolated Perfused Dog Pancreas[1] (39025)

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Evidence for neurogenic control of insulin secretion mediated by the autonomic nervous system has been provided by numerous studies (1). The sympathetic autonomic division is believed to exert an inhibitory influence upon insulin release either by direct fiber innervation at the level of the β-cell (2), or following the release of amines from the adrenal medulla (3, 4). Stimulation of the mixed nerve supply to the pancreases of atropinized dogs has been shown to block glucose-induced insulin secretion (2), and basal as well as stimulated insulin secretions have been reported to be depressed following administration of epinephrine and norepinephrine both *in vivo* (3–5) and *in vitro* (6, 7).

In contrast to the inhibitory influence exerted by sympathetic activation, increased cholinergic activity is associated with an enhanced insulin release (1). Electrical stimulation of the parasympathetic limb of the autonomic nervous system at the level of mixed pancreatic nerves (2), the vagal trunks (8), or the motor nuclei of the vagus (9) elicits insulin secretion, and the parasympathetic mediator, acetylcholine, has been thoroughly documented as a potent *in vitro* insulinotropic agent whose secretory effect can be inhibited by atropine and potentiated by cholinesterase (6, 10, 11).

In this report, the insulinotropic actions of glucose and acetylcholine are compared in the isolated, perfused dog pancreas, and the secretory response to acetylcholine in this preparation is characterized as a muscarinic action of the agent which is dependent upon the microtubular system of the β-cell.

Methods and Materials. Mongrel dogs of both sexes weighing 18–28 kg were used. All animals were fasted overnight with free access to water. The procedure for isolation and perfusion of the dog pancreas has been described previously (12). Acetylcholine chloride (Pflatz and Bauer, Inc.), atropine sulfate (Nutritional Biochemicals Co.), and colchicine (Sigma Chemical Co.) were added as required to the normal perfusate. Insulin secretion is expressed as μU/min as calculated from the flow rate and concentration of insulin in the effluent. The Student's two sample *t* test was used for all statistical comparisons.

Results. Prior to time zero, each pancreas was perfused for 20–30 min with normal medium to establish a basal secretory rate (<500 μU/min). Unless otherwise stated, the results described hereafter refer to the period from time zero to the completion of the experiment.

Perfusion with a high glucose concentration (300 mg%) for 60 min resulted in a biphasic release of insulin (Fig. 1). The first phase was characterized by a rapid increase in secretion wich reached a peak during the third and fourth minutes of simulation and then declined until min 12. The second phase of secretion was characterized by an increasing rate of insulin release from min 12 until the termination of the perfusion at min 60.

In six separate experiments 50 μ*M* acetylcholine was perfused for a 60-min interval (Fig. 1). Introduction of this agent caused an immediate increase in secretion which was more monophasic than biphasic in nature. Peak secretion was delayed until approximately 10 min after the start of the perfusion and, in contrast to glucose-induced insulin release, was noted to decrease during the latter part of the 60 minute perfusion. In two of the six acetylcholine perfusions, termination of the acetylcholine pulse was followed by a 10-min control period preceding a 5-min 300 mg% glucose challenge. In both in-

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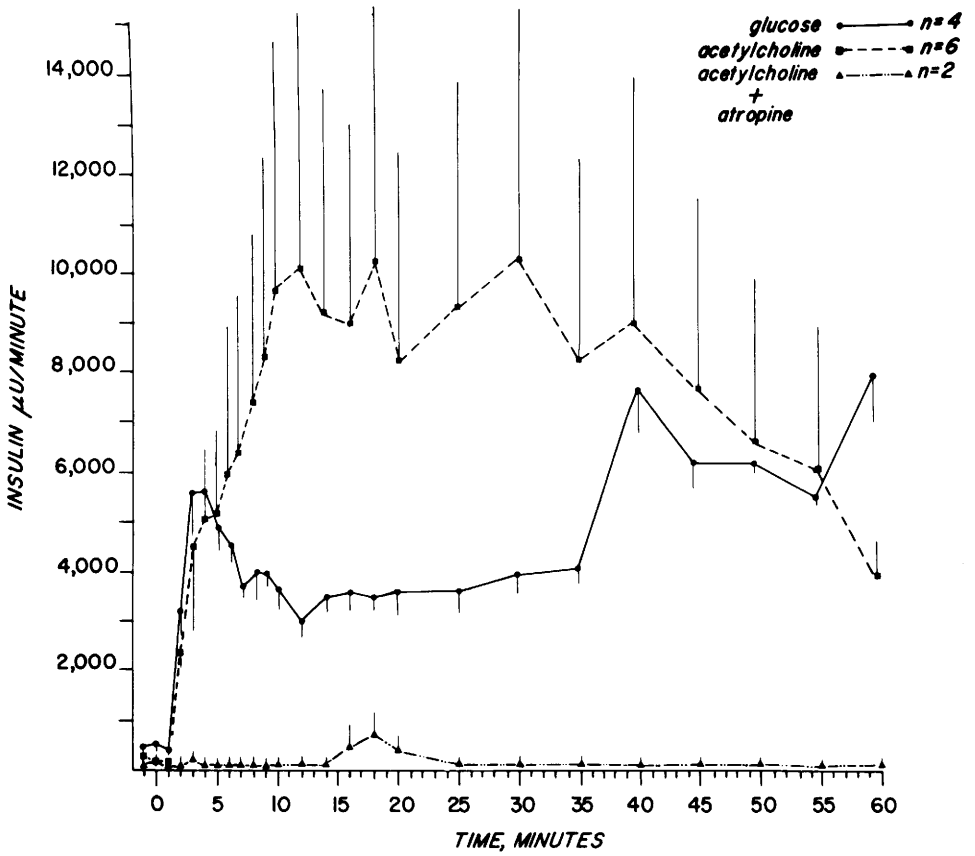

FIG. 1. Stimulation of insulin secretion by 300 mg% glucose ($N = 4$) and 50 μM acetylcholine ($N = 6$), and inhibition of 50 μM acetylcholine-induced insulin release by 25 μM atropine ($N = 2$). Perfusates containing high glucose or acetylcholine were introduced at zero time and continued for 60 min. Atropine was started 10 min prior to time zero and continued for 70 min. Each value represents the mean ± SEM.

stances (Table I) this glycemic stimulus elicited an increase in insulin output which reached values six times greater than that seen at min 60 of the 50 μM acetylcholine perfusion.

The potent insulinotropic action of acetylcholine was blocked by 25 μM atropine (Fig. 1). Perfusion with atropine for 10 min prior to and during the acetylcholine exposure almost totally abolished secretion. Only a small, brief output of insulin occurred in one of two atropine experiments (min 14–25).

The mitotic spindle inhibitor, colchicine, was perfused from min 5 to 115 at a concentration of 1 mM in a group of experimental pancreases which received 50 μM acetylcholine from min 0 to 5 and 95 to 115 (Fig. 2). The results of these experiments were compared to a set of control pancreases (Fig. 2) which received only 50 μM acetylcholine from min 0 to 5 and 95 to 115. No significant difference was noted between the secretion elicited by the first five minute

TABLE I. GLUCOSE-INDUCED INSULIN SECRETION FOLLOWING CONTINUOUS INFUSION OF ACETYLCHOLINE (ACh) IN TWO PERFUSIONS.

Time (min)	Experimental condition	Insulin (μmicrounits/min) PP[a] 276	PP[a] 278
60	50 μM ACh	1905	4858
65	Control	3450	3954
70	Control	2145	2352
75	300 mg% glucose	12330	28532

[a] PP = pancreas perfusion number.

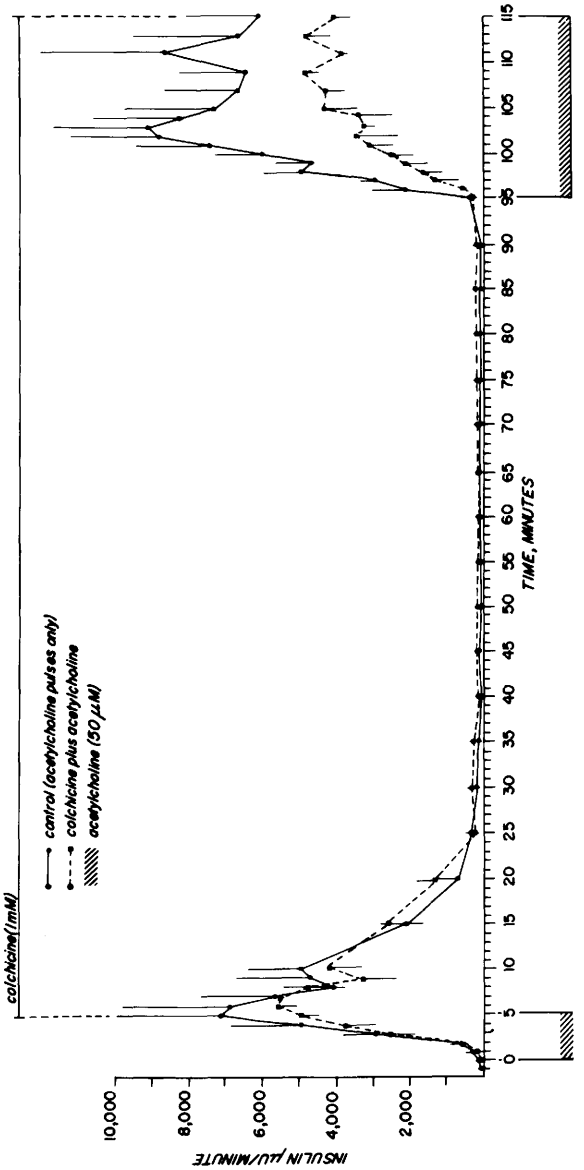

FIG. 2. Inhibition of acetylcholine-induced (50 μM) insulin secretion by colchicine (1 mM). The solid line represents the mean ± SEM of seven control experimnts in which acetylcholine was administered between min 0 to 5 and 95 to 115. The dashed line represents the mean ± SEM of eight colchicine experiments in which colchicine was present 90 min prior to and during the second acetylcholine pulse.

pulse of acetylcholine in the control and experimental pancreases, but the release stimulated by the second acetylcholine pulse (min 95–115) in the experimental group was significantly lower ($P < 0.05$) than that of the control group.

Discussion. In the present experiments 60 mg% glucose was used in the buffer medium for all prestimulatory, control, and acetylcholine perfusion periods. When this nonstimulatory concentration of glucose was followed by perfusion with buffer containing 300 mg% glucose, a typical biphasic pattern of insulin secretion (13) was observed. In contrast, perfusion with 50 μM acetylcholine consistently resulted in a potent monophasic insulin secretory response by the isolated dog pancreas. Other investigators have noted a biphasic release of insulin from isolated, perfused rat and dog pancreas preparations following acetylcholine administration, but only when stimulatory glucose concentrations (150 mg%) were present in the perfusate (10, 11).

The decrease in secretory rate during the latter part of acetylcholine perfusions in the current experiments is in agreement with data obtained in the perfused rat pancreas preparation (11) where it was found that acetylcholine-induced insulin secretion declined during a 30-min perfusion period to below prestimulatory values. The sixfold increase in insulin output that was elicited by perfusion with 300 mg% glucose following the termination of acetylcholine perfusion in two dog pancreases suggests that the decrease in insulin secretion noted with continued exposure to acetylcholine is not due to β-cell exhaustion. From data obtained in other studies, a more plausible explanation is that desensitization to acetylcholine develops upon long-term administration (14).

Atropine was found to almost totally abolish acetylcholine-stimulated insulin release from the isolated dog pancreas in two experimental perfusions. This data confirms the inhibitory nature of atropine under the experimental conditions used in the present study, and adds support to the current concept of the dependence upon muscarinic receptors for the secretory action of acetylcholine at the level of the β-cell (6, 10, 11).

The insulinotropic action of acetylcholine is highly dependent upon extracellular calcium, and it can be suggested that movement of calcium across the plasma membrane of the β-cell may be a key event in this process (12). Since an increase in β-cell cytoplasmic Ca^{2+} is thought to play a major role in the insulin secretory process by activating the microtubular system of the β-cell (15), the mitotic spindle inhibitor, colchicine, was tested as a possible inhibitor of acetylcholine's action. When compared to control values, perfusion with 1 mM colchicine resulted in a 50% inhibition of insulin secretion. This suggests that the insulinotropic action of acetylcholine is dependent, at least in part, upon a normally functioning microtubular system. The lack of total inhibition may be due to a dosage related problem (16) or may be representative of a secretory action of acetylcholine which is independent of the microtubular system of the β-cell. Although colchicine and other mitotic spindle inhibitors such as vincristine and vinblastine have been thoroughly documented as inhibitors of glucose-induced insulin secretion (16–18), the effect of these agents upon acetylcholine-stimulated insulin release has not been previously tested. However, in the adrenal medulla catecholamine secretion elicited by acetylcholine has been similarly shown to be inhibited by only approximately 50% in the presence of colchicine (19).

Summary. In the presence of a nonstimulatory concentration of glucose, a 60-min perfusion with 50 μM acetylcholine was shown to elicit a monophasic release of insulin in the isolated dog pancreas preparation. A decline in secretory response, which may be due to desensitization of the β-cell to acetylcholine, was noted during the latter part of the perfusion interval. The potent insulin secretory response elicited by acetylcholine during the 60-min period was abolished by 25 μM atropine. Inhibition of the insulinotropic action of acetylcholine was also noted with administration of the mitotic spindle inhibitor, colchicine. When compared to 20-min control perfusions, addition of 1 mM colchicine resulted in a 50% reduction in acetylcholine-induced insulin release. These results suggest that insulin secretion

stimulated by acetylcholine can be considered to be due to a muscarinic action of this agent which is dependent, at least in part, upon the microtubular system of the β-cell.

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