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INHIBITION BY MEPACRINE AND *p*-BROMOPHENACYLBROMIDE OF PHOSPHOINOSITIDE HYDROLYSIS, GLUCOSE OXIDATION, CALCIUM UPTAKE AND INSULIN RELEASE IN RAT PANCREATIC ISLETS

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Abstract—Mepacrine and *p*-bromophenacylbromide were both found to impair ^{3}H-inositol phosphate production in response to both nutrient and hormone-neurotransmitter stimuli in islets prelabelled with ^{3}H-inositol. Both drugs also inhibited net ^{45}Ca uptake in response to glucose or glibenclamide and considerably modified the patterns of ^{45}Ca and ^{86}Rb efflux from perifused islets under both basal and glucose-stimulated conditions. In addition, the oxidation of [U-^{14}C] glucose in islets was impaired by either mepacrine or *p*-bromophenacylbromide. These inhibitory effects were found to be concentration-related for both mepacrine (0.01–1.0 mM) and *p*-bromophenacylbromide (0.03–0.3 mM) and were accompanied, in general, by a similar degree of inhibition of insulin secretion. These results suggest that both mepacrine and *p*-bromophenacylbromide can inhibit phospholipase C activity in intact islets, but also impair ^{45}Ca and ^{86}Rb fluxes and oxidation of nutrients. The diversity of these drugs' inhibitory actions makes them unsuitable tools for examining the role of specific cellular processes in the regulation of islet function.

The ability of a number of drugs to inhibit various aspects of cell function has been ascribed to their apparent activity as inhibitors of phospholipase A_2. This enzyme, which deacylates fatty acids at the 2-position of the phospholipid, is thought to play an essential role in the liberation of arachidonic acid for the subsequent formation of prostaglandins, thromboxanes and lipoxygenation products [1]. Thus, it is generally accepted that drugs such as mepacrine (quinacrine) and *p*-bromophenacylbromide, which inhibit the production of arachidonic acid and its active metabolites, do so by inhibiting phospholipase A_2 activity [2–6]. Nevertheless, the further possibility exists that drugs of this type may also inhibit phospholipid breakdown by a phospholipase C mechanism [7, 8] which is an important step in the regulation of the so-called phosphatidylinositol cycle [9].

We have shown in the present study that both mepacrine and *p*-bromophenacylbromide result in an apparent inhibition of phospholipase C activity in intact rat pancreatic islets, as measured by the production of ^{3}H-inositol phosphates in ^{3}H-inositol prelabelled islets [10]. However, these drugs also exert powerful inhibitory actions upon other parameters of islet function, namely ^{14}C-glucose oxidation, ^{45}Ca net uptake and insulin secretion. Therefore, such compounds are of limited value in assessing the role of phospholipases in intact cells.

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MATERIALS AND METHODS

Pancreatic islets were isolated from fed adult rats by collagenase digestion [11]. For studies of ^{3}H-inositol phosphate production, groups of islets were incubated in glass tubes in 1.0 ml gassed bicarbonate medium [12] containing 2.8 mM glucose, 0.25% (w/v) bovine serum albumin and 6 μCi myo-^{3}H-inositol for 2 hr at 37°. The medium was then removed and replaced by 0.9 ml fresh, unlabelled medium containing 5 mM LiCl, 1 mM inositol and appropriate concentrations of mepacrine of *p*-bromophenacylbromide. The islets were then incubated for 10 min at 37°, 100 μl medium containing test substances was added, and the incubations were continued for a further 20 min. Incubations were terminated by the addition of 3 ml $CHCl_3$:MeOH (2:1 v/v), the tubes were vortex-mixed for 30 sec and subsequently centrifuged to separate the two phases. The upper, aqueous phase was removed, mixed with 2 ml H_2O and applied to a 0.5 ml column of Dowex AG1X8 (200–400) formate form. Following elution of the free inositol with 4 × 2.5 ml H_2O, inositol phosphates were eluted with 2 × 1 ml 1 M ammonium formate/0.1 M formic acid. Aquasol scintillant (10 ml) was added to each fraction and the sample counted for radioactivity.

The methods used to measure ^{45}Ca net uptake [13], ^{45}Ca efflux [14], ^{86}Rb efflux [15], [U-^{14}C]glucose oxidation [16] and insulin secretion [12] in islets have been described in detail elsewhere.

Myo-[2-^{3}H]inositol (16.4 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, U.K. Carbamylcholine, cholecystokinin-pancreozymin, mepacrine (quinacrine) dihydrochloride and *p*-

bromophenacylbromide were purchased from Sigma Chemical Co., St Louis, Missouri. *p*-Bromophenacylbromide was dissolved in dimethylsulfoxide, the final concentration of which did not exceed 1% (v/v). At this concentration, no effect of dimethylsulfoxide was apparent either on ^{3}H-inositol phosphate production or on other parameters of islet function under study.

All results are expressed as the mean (± S.E.M.) together with the number of individual determinations (in parentheses). The statistical significance of differences between mean values was assessed by use of the Student's *t*-test.

RESULTS

The stimulation of ^{3}H-inositol-prelabelled islets with glucose (22.2 mM), carbamylcholine (2 mM) or cholecystokinin-pancreozymin (1 u/ml) resulted in a marked production of ^{3}H-inositol phosphates (Table 1). In all cases, this response was inhibited by preincubating the islets for 10 min with either mepacrine (1 mM) or *p*-bromophenacylbromide (300 μM). The inhibition of ^{3}H-inositol phosphate production by these compounds was found to be concentration-dependent whether glucose (Fig. 1) or another stimulus was used. In glucose-stimulated islets, a 50% inhibition was seen at concentrations close to 0.06 mM for mepacrine and 0.1 mM for *p*-bromophenacylbromide.

Mepacrine was also found to produce a concentration-dependent inhibition of net ^{45}Ca uptake into islets in response to 16.7 mM glucose and to 50 μg/ml glibenclamide (Tables 2 and 3). In the case of *p*-bromophenacylbromide, however, a significant inhibition of glucose-stimulated ^{45}Ca net uptake was only found at the highest concentration of the drug (Table 2). Figure 2 illustrates the effect of mepacrine and *p*-bromophenacylbromide upon ^{86}Rb and ^{45}Ca outflow from prelabelled islets exposed to a square-waved increase in glucose concentration from zero to 16.7 mM. Prior to glucose administration, mepacrine considerably decreased the basal ^{86}Rb FOR (min 31 to 45) from a control value of 3.10 ± 0.20 to 1.07 ± 0.32%/min ($P < 0.001$), and slightly decreased the basal ^{45}Ca FOR from a control value of 1.09 ± 0.04 to 0.73 ± 0.19%/min ($P < 0.05$). *p*-

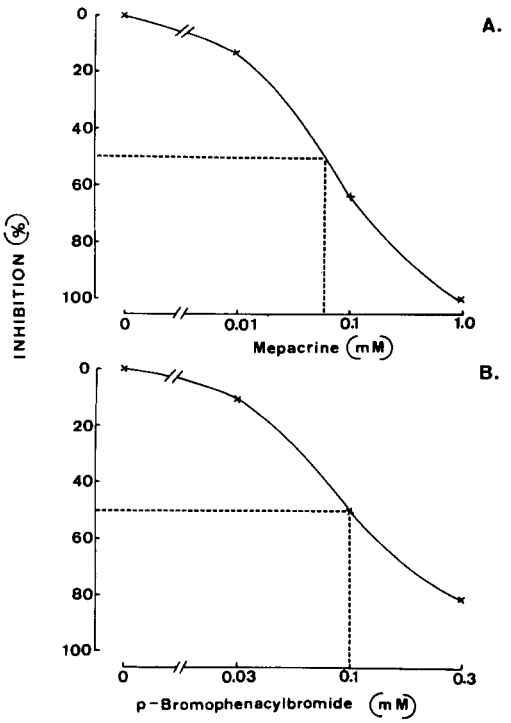

Fig. 1. Dose-dependent inhibition of ^{3}H-inositol phosphate production in response to 22.2 mM glucose by mepacrine (A) and by *p*-bromophenacylbromide (B). Each point represents the mean of two observations. The dashed lines refer to a 50% inhibition.

Bromophenacylbromide, on the contrary, increased slightly the basal ^{86}Rb FOR to 3.77 ± 0.19%/min ($P < 0.05$) and markedly augmented the basal ^{45}Ca FOR to 1.57 ± 0.09%/min ($P < 0.001$). Several further anomalies were observed in response to glucose administration. In the presence of mepacrine, glucose, instead of causing a sustained decrease in ^{86}Rb FOR, provoked a progressive, albeit modest, increase in ^{86}Rb outflow. In the presence of *p*-bromophenacylbromide, glucose caused the usual decrease in ^{86}Rb FOR, but the mean value reached between the 56th and 70th min of perifusion (2.01 ± 0.18%/min) remained higher ($P < 0.005$)

Table 1. Effects of mepacrine and *p*-bromophenacylbromide (BPB) on ^{3}H-inositol phosphate production in rat islets in response to various secretagogues

Line	Secretagogue	Mepacrine (1 mM)	BPB (300 μM)	^{3}H-inositol phosphate (cpm/150 islets)	P
1	Control (2.8 mM glucose)	−	−	873 ± 96	
2	22.2 mM glucose	−	−	4873 ± 739	vs line 1, $P < 0.005$
3	22.2 mM glucose	+	−	539 ± 28	vs line 2, $P < 0.0025$
4	22.2 mM glucose	−	+	727 ± 89	vs line 2, $P < 0.005$
5	2 mM carbamylcholine	−	−	4004 ± 448	vs line 1, $P < 0.0025$
6	2 mM carbamylcholine	+	−	653 ± 139	vs line 5, $P < 0.0025$
7	2 mM carbamylcholine	−	+	758 ± 56	vs line 5, $P < 0.0025$
8	1 u/ml pancreozymin	−	−	3075 ± 400	vs line 1, $P < 0.01$
9	1 u/ml pancreozymin	+	−	342 ± 166	vs line 8, $P < 0.0025$
0	1 u/ml pancreozymin	−	+	639 ± 452	vs line 8, $P < 0.01$

Incubations were for 20 min. Each value represents the mean ± S.E. of three separate determinations.

Table 2. Effects of mepacrine and *p*-bromophenacylbromide (BPB) on ^{45}Ca net uptake in response to glucose

Line	D-glucose (mM)	Mepacrine (mM)	BPB (mM)	^{45}Ca net uptake (pmol/islet at 90 min)	P
1	Nil	—	—	1.87 ± 0.13 (12)	vs line 2 < 0.001
2	16.7	—	—	4.53 ± 0.17 (49)	
3	16.7	0.01	—	4.11 ± 0.20 (26)	vs line 2 N.S.
4	16.7	0.10	—	2.32 ± 0.17 (27)	vs line 2 < 0.001
5	16.7	1.00	—	0.47 ± 0.02 (11)	vs line 2 < 0.001
6	16.7	—	0.03	4.39 ± 0.15 (10)	vs line 2 N.S.
7	16.7	—	0.10	4.85 ± 0.14 (22)	vs line 2 N.S.
8	16.7	—	0.30	1.89 ± 0.13 (10)	vs line 2 < 0.001

The figures in parentheses represent the number of individual determinations.
N.S.: not significant.

than that reached at the same time in the control experiments (1.32 ± 0.06%/min). Both mepacrine and *p*-bromophenacylbromide impaired the capacity of glucose to cause an early fall in ^{45}Ca FOR. Indeed, the difference between the value reached at the 45th min, just prior to glucose administration, and the lowest mean value recorded shortly thereafter averaged 0.12 ± 0.06 and 0.21 ± 0.05%/min in the presence of mepacrine and *p*-bromophenacylbromide, respectively, as distinct from 0.39 ± 0.02%/min in the control experiments ($P < 0.005$ in both cases). Likewise, both mepacrine and *p*-bromophenacylbromide altered the capacity of glucose to cause a secondary rise in ^{45}Ca FOR. Thus, between the 50th

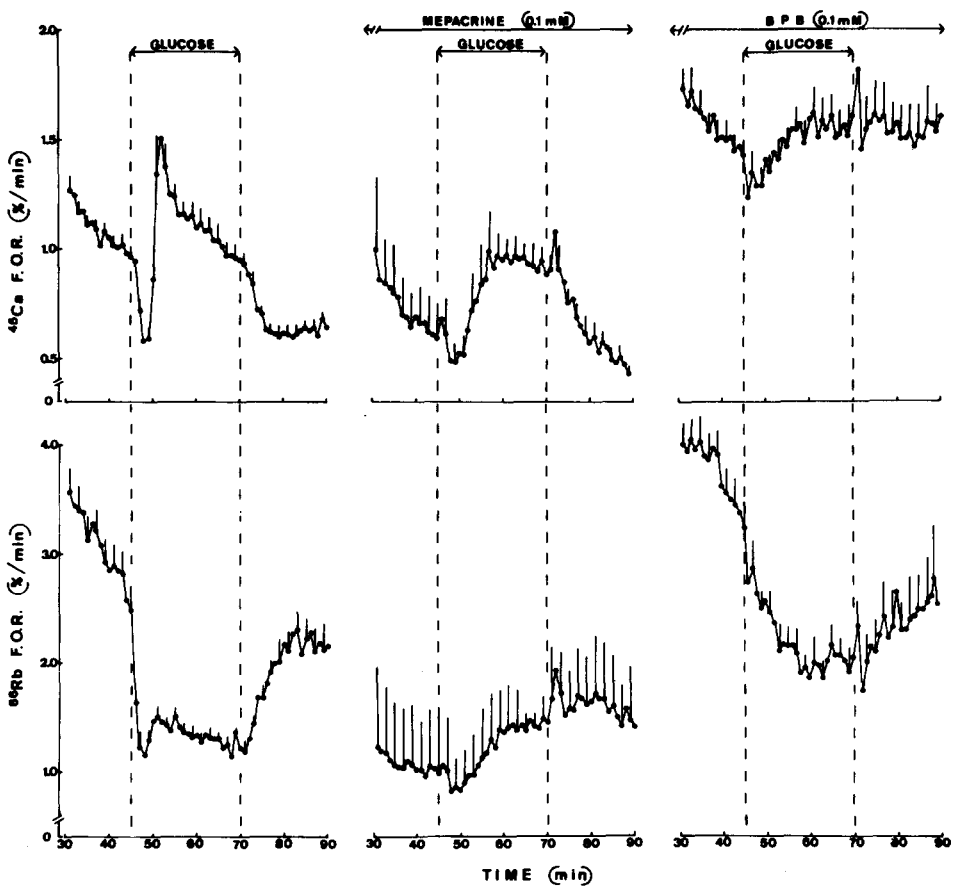

Fig. 2. Effect of a rise in glucose concentration from zero to 16.7 mM (between the 46th and 70th min) upon ^{45}Ca FOR (upper panel) and ^{86}Rb FOR (lower panel) from islets perifused for 90 min in the absence (left panels) or presence of either mepacrine (middle panels) or *p*-bromophenacylbromide (right panels). Mean values (± S.E.M.) refer to four (middle panels) or eight (right and left panels) individual experiments.

Table 3. Effects of mepacrine and *p*-bromophenacylbromide (BPB) on ^{45}Ca net uptake in response to glibenclamide

Line	Glibenclamide (μg/ml)	Mepacrine (mM)	BPB (mM)	^{45}Ca net uptake (pmol/islet at 90 min)	P
1	Nil	—	—	2.19 ± 0.24 (11)	
2	50	—	—	5.40 ± 0.21 (12)	vs line 1, $P < 0.001$
3	50	0.1	—	1.68 ± 0.13 (12)	vs line 2, $P < 0.001$
4	50	1.0	—	0.61 ± 0.03 (6)	vs line 2, $P < 0.001$
5	50	—	0.1	3.94 ± 0.37 (12)	vs line 2, $P < 0.001$
6	50	—	0.3	2.06 ± 0.05 (12)	vs line 2, $P < 0.001$

The figures in parentheses represent the number of individual determinations.

Table 4. Effects of mepacrine and *p*-bromophenacylbromide (BPB) upon oxidations of D-[U-^{14}C]glucose by pancreatic islets

Line	D-[U-^{14}C]glucose (mM)	Mepacrine (mM)	BPB (mM)	Oxidation (pmol/120 min per islet)	P
1	16.7	—	—	29.3 ± 1.4 (47)	
2	16.7	0.01	—	33.5 ± 1.1 (25)	vs line 1, $P < 0.01$
3	16.7	0.10	—	16.7 ± 1.1 (26)	vs line 1, $P < 0.001$
4	16.7	1.00	—	3.4 ± 0.4 (20)	vs line 1, $P < 0.001$
5	16.7	—	0.03	23.9 ± 1.1 (9)	vs line 1, N.S.
6	16.7	—	0.10	11.8 ± 2.1 (10)	vs line 1, $P < 0.001$
7	16.7	—	0.30	7.4 ± 2.4 (9)	vs line 1, $P < 0.001$

The figures in parentheses represent the number of individual determinations.
N.S.: not significant.

Table 5. Effects of mepacrine and *p*-bromophenacylbromide (BPB) upon insulin output from pancreatic islets

Line	Secretagogue(s) (mM)	Mepacrine (mM)	BPB (mM)	Insulin output (μU/90 min per islet)	P
1	Nil	—	—	20.7 ± 2.3 (11)	
2	D-glucose 16.7	—	—	256.4 ± 12.1 (37)	
3	D-glucose 16.7	0.01	—	284.1 ± 15.1 (18)	vs line 2, N.S.
4	D-glucose 16.7	0.10	—	46.2 ± 4.6 (18)	vs line 2, $P < 0.001$
5	D-glucose 16.7	1.00	—	32.6 ± 6.2 (18)	vs line 2, $P < 0.001$
6	D-glucose 16.7	—	0.03	294.1 ± 35.4 (19)	vs line 2, N.S.
7	D-glucose 16.7	—	0.10	148.2 ± 19.5 (19)	vs line 2, $P < 0.001$
8	D-glucose 16.7	—	0.30	96.7 ± 10.5 (19)	vs line 2, $P < 0.001$
9	2-ketoisocaproate 10	—	—	55.0 ± 5.3 (9)	
10	2-ketoisocaproate 10	0.10	—	28.6 ± 3.1 (9)	vs line 9, $P < 0.001$
11	L-glutamine 10 + L-leucine 10	—	—	126.9 ± 14.5 (9)	
12	L-glutamine 10 + L-leucine 10	0.10	—	25.2 ± 5.7 (9)	vs line 11, $P < 0.001$
13	$BaCl_2$ 2 + theophylline 1.4	—	—	67.9 ± 3.6 (11)	
14	$BaCl_2$ 2 + theophylline 1.4	0.10	—	27.2 ± 2.3 (12)	vs line 13, $P < 0.001$

The figures in parentheses represent the number of individual determinations. N.S.: not significant. The effect of Ba^{2+} and theophylline was tested in the absence of Ca^{2+}.

and 70th min of perifusion, the glucose-induced increment in ^{45}Ca FOR in excess of the value reached at the 48th to 49th min averaged 0.37 ± 0.06 and 0.23 ± 0.04%/min in the presence of mepacrine and *p*-bromophenacylbromide, respectively, as distinct from 0.53 ± 0.04%/min in the control experiments ($P < 0.05$ and $P < 0.001$, respectively).

Both inhibitors were found to impair oxidation of D-[U-^{14}C]glucose in islets (Table 4). These effects were again concentration-dependent for both drugs, although no inhibition of glucose oxidation was noted at the lower concentrations used; on the contrary, mepacrine (0.01 mM) was found to cause a slight though significant enhancement of glucose oxidation.

The effects of mepacrine and *p*-bromophenacylbromide on insulin secretion are summarized in Table 5. In the case of mepacrine, no effect was observed at the lower concentration (0.01 mM), al-

though the higher concentrations (0.1 and 1 mm) resulted in almost complete inhibition of stimulated insulin release. Likewise, *p*-bromophenacylbromide caused a concentration-related inhibition of insulin secretion, although the highest concentration of the drug used (300 μM) only inhibited glucose-stimulated insulin release by approximately 70%. Mepacrine also inhibited insulin release evoked by a variety of stimuli, including 2-ketoisocaproate, and combinations of either glutamine with leucine or barium chloride with theophylline (Table 5).

DISCUSSION

The stimulation by certain secretagogues of ^{3}H-inositol phosphate production in islets prelabelled with ^{3}H-inositol was the subject of a previous study from this laboratory [10] and implies accelerated hydrolysis of inositol-containing lipids by a phospholipase C mechanism [17]. This technique is, therefore, especially suitable for examining the effects of drugs on phospholipase C activity in intact cells, particularly compounds such as mepacrine which have been shown to bind to certain lipids and alter their mobility in chromatographic systems [18]. The inositol phosphates measured in the present study are water-soluble and are unlikely to be affected by such interactions. The results obtained suggest that both mepacrine and *p*-bromophenacylbromide, purported inhibitors of phospholipase A_2 activity [2–6], also possess activity as inhibitors of phospholipase C, at least in pancreatic islets stimulated by nutrient or hormone-neurotransmitter stimuli. In view of the possibility that phospholipase inhibition by certain compounds may arise from an interaction with the substrate (as well as, or instead of interaction with the enzyme [6, 7, 18]), it is perhaps predictable that such drugs may produce a non-specific inhibition of phospholipases A_2 and C. Furthermore, a number of investigation have provided evidence that the cellular effects of mepacrine and *p*-bromophenacylbromide are not limited to inhibition of phospholipase activities. In particular, Tsai and colleagues [19] have reported that mepacrine inhibits the binding of ^{3}H-acetylcholine to its receptor in membranes from *Torpedo* electric organ. This finding may at least in part explain the inhibition by mepacrine of carbamylcholine-stimulated phosphoinositide hydrolysis described in this paper. The effects of mepacrine (or *p*-bromophenacylbromide) have not, as far as we are aware, been investigated upon the binding of other agonists such as pancreozymin. However, our subsequent studies with islets of Langerhans have revealed a number of inhibitory effects of mepacrine and *p*-bromophenacylbromide which are unlikely to result simply from inhibition of phospholipase C (or A_2) activity. A marked effect of both drugs was an inhibition of net ^{45}Ca uptake in islets. In view of the hypothesis originally put forward by Michell [20] that enhanced inositol lipid turnover during cell stimulation may be involved in the regulation of calcium mobilization, it might be expected that drugs which inhibit phospholipases would also impair calcium handling by the cell. However, the fact that both mepacrine and *p*-bromophenacylbromide inhibited calcium uptake in response to glibenclamide, a secretagogue which does not apparently induce phosphoinositide hydrolysis [10], suggests that these drugs exert a more general inhibitory effect on calcium mobilization, not solely through impaired phospholipid metabolism. It is therefore interesting to note that both drugs have previously been found to inhibit calcium uptake and secretion in adrenal medullary cells in response to nicotinic agonists, K^+, Ba^{2+} and ionophore A23187 [21].

The effects of mepacrine and *p*-bromophenacylbromide upon ^{86}Rb and ^{45}Ca outflow from prelabelled islets further document the fact that these drugs do not act as specific phospholipase inhibitors in the islet cells. In the case of mepacrine, the lower basal ^{86}Rb FOR and the fact that glucose augmented ^{86}Rb FOR are reminiscent of the situation found in islets exposed to quinine (0.1 mM), which is presumed to act as a blocker of the Ca^{2+}-activated K^+ channel [22]. In the islets exposed to mepacrine, the pattern of ^{45}Ca efflux in the basal state as well as in response to glucose stimulation was also similar to that observed in islets exposed to quinine. In both cases, the initial peak normally characterizing the secondary rise in ^{45}Ca FOR was virtually abolished, but a late stimulation of ^{45}Ca FOR by glucose was nevertheless evident. In the case of *p*-bromophenacylbromide, the ^{86}Rb response to glucose was grossly normal, except for a somewhat higher absolute value for ^{86}Rb FOR both in the basal state and during glucose stimulation. *p*-Bromophenacylbromide, however, augmented the basal ^{45}Ca FOR and markedly impaired the ^{45}Ca dual response to glucose. These findings could be taken as an indication that *p*-bromophenacylbromide alters the intracellular distribution of ^{45}Ca, as also suggested by the fact that *p*-bromophenacylbromide (0.1 mM) impaired glucose-induced insulin release despite an apparently normal net uptake of ^{45}Ca by the islets.

An additional inhibitory effect of mepacrine and *p*-bromophenacylbromide was upon the oxidation of glucose in islets. Such an effect was observed at concentrations of the drugs which inhibited inositol lipid breakdown and ^{45}Ca handling, and it is possible that an inhibition of glucose oxidation could contribute towards the two latter effects. However, at the high concentration of glucose (22.2 mM) used in the lipid experiments, an approximately 50% inhibition of oxidation by either drug would still result in a rate of glucose metabolism which would be expected to exert a near-maximal effect upon inositol lipid turnover [23]. Since the latter was, in fact markedly reduced under these conditions, it seems likely, at least on theoretical grounds, that mepacrine and *p*-bromophenacylbromide can impair inositol lipid hydrolysis by a mechanism other than the inhibition of glucose oxidation. It is thus conceivable that the inhibition by mepacrine and *p*-bromophenacylbromide of insulin secretion, which was observed with all of the secretagogues examined, could be a result of impaired inositol lipid turnover, calcium uptake and distribution or nutrient metabolism in the islet, or a combination of these effects. The non-specificity of these drugs has been previously noted in a number of biological systems [8, 19, 21, 24, 25] and the present study further emphasizes the need

to exercise caution in interpreting data obtained from their use. To say the least, it would seem premature to ascribe the stimulation [26] or inhibition [27] of insulin release by mepacrine, as reported in prior publications, to a specific inhibition of phospholipase A_2 activity.

In conclusion, we have found evidence that both mepacrine and *p*-bromophenacylbromide can act as inhibitors of phospholipase C in intact islets. However, in attempting to correlate this inhibitory activity with other aspects of islet function, we have demonstrated a number of additional effects of the drugs, all of which could contribute towards an impairment of insulin secretion. This non-specificity of mepacrine and *p*-bromophenacylbromide makes these drugs unsuitable as probes for the investigation of the role of processes such as enhanced inositol phospholipid turnover in the regulation of islet function.

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