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CALCIUM-ANTAGONISTS AND ISLET FUNCTION—XII. COMPARISON BETWEEN NIFEDIPINE AND CHEMICALLY RELATED DRUGS

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Abstract—The effects of nifedipine and four chemically related drugs upon insulin release by isolated rat islets were compared. All drugs inhibited glucose-induced insulin release. The dose–action relationship yielded comparable slopes, each ten-fold increase in drug concentration causing a 42.1 ± 1.1 per cent fall in insulin output. However, the ED_{50} for the inhibitory effects of the drug ranged from $0.99 \cdot 10^{-8}$ M (Bay k 5552) to $0.84 \cdot 10^{-7}$ M (Bay a 1040). Total suppression of glucose-stimulated insulin release was invariably seen at a 10^{-6} M concentration of each drug. It is concluded that, despite significant variation in its potency, a calcium-antagonistic property is shared by nifedipine and several structurally related compounds.

In the preceding reports in this series [1–11], we have characterized the influence of various inorganic and organic calcium-agonists and antagonists upon different parameters of islet function. In the case of organic calcium-antagonists such as verapamil, suloctidil and nifedipine, the experimental data suggested that these drugs cause a rather selective inhibition of calcium entry into the islet cells and, by doing so, provoke a rapid, sustained and dose-related inhibition of insulin release evoked by glucose or other secretagogues.

The finding that these organic calcium-antagonists also inhibit the stimulant action of the calcium-ionophore A23187 upon insulin release [12] led us to postulate that these drugs may interfere with the capacity of both the antibiotic ionophore and native ionophoretic system present in the plasma membrane of islet cells to translocate calcium across a hydrophobic domain. This view is supported by recent observations on the effect of verapamil and suloctidil upon ionophore-mediated calcium translocation in artificial systems [13–16].

The relative potency of distinct organic calcium-antagonists to inhibit insulin relase and to interfere with other calcium-dependent physiological processes (e.g. smooth muscle contraction), respectively, is of obvious importance in the design of pharmaceutical agents specifically directed towards one or the other of these calcium-dependent processes. In such a perspective, we have here compared the effect of nifedipine to that of four chemically related drugs upon insulin release, with emphasis on the relative potency of each drug.

MATERIAL AND METHODS

Insulin release was measured in groups of 8 islets each incubated in 1.0 ml of medium, as described elsewhere [17]. The islets were first collected in 0.5 ml of incubation medium containing no glucose and no drug. The volume of the medium was then increased to 1.0 ml by addition of 0.5 ml of a medium containing glucose and the drug to be tested. The latter addition and further incubation (90 min) were performed in a dark room equipped with a sodium vapour lamp, in order to prevent the inactivation of the drug. The final concentration of glucose in the incubation medium was 16.7 mM in all cases. All drugs were initially dissolved in dimethylsulphoxide at a 2 mM concentration. This solution was then added to the incubation medium to give final drug concentrations ranging from 10^{-9} to 10^{-6} M. The highest final concentration of dimethylsulphoxide amounted to 5.0 μl/ml (v/v); at this concentration, the solvent exerts no effect upon insulin release and glucose metabolism in the islets [18]. Table 1 provides the chemical structure of the five drugs examined in the present study.

Table 1. Chemical structure of the drugs under study

Line	Compound	R_1	R_2	R_3
(a)	Bay a 1040	-(*o*-nitro-phenyl)	-CO—O—CH_3	-CO—O—CH_3
(b)	Bay e 5009	-(*m*-nitro-phenyl)	-CO—O—CH_3	-CO—O—C_2H_5
(c)	Bay k 9320	-(*o*-chloro-phenyl)	-CO—O—CH_2—CF_3	-CO—O—CH_3
(d)	Bay k 5552	-(*o*-nitro-phenyl)	-CO-O-CH_3	-CO-O-CH_2-$CH_2CH_3)_2$
(3)	Bay e 9736	-(*m*-nitro-phenyl)	-CO—O—$(CH_2)_2$—O—CH_3	-CO—O—$CH(CH_3)_2$

* All drugs were derived from 1,4-dihydro-2,6-dimethyl-4-R_1-3-R_2-5-R_3-pyridine, formula in which R_1, R_2 and R_3 stand for the corresponding radicals shown in the table [19–22]. All drugs were provided by Bayer AG (Wuppertal, FRG).

In all experiments, equal numbers of determinations were performed in the absence and presence of each concentration of each drug. The dose-related effect of each drug was tested in separate experiments (see Fig. 1). However, in one series of experiments, the effects of all drugs were directly compared within the same experiments, all drugs being tested at the same concentration (7.10^{-8} M; see Table 2). All results are expressed as the mean (± SEM) together with the number of individual observations (n). Insulin output is expressed in per cent of the mean control value found within the same experiment in media containing only glucose.

RESULTS

In the presence of glucose 16.7 mM, the control value for insulin output averaged 218.9 ± 6.5 μU/90 min per islet (n = 114). At a 10^{-9} M concentration, all drugs only caused marginal changes in insulin output, the overall mean value for insulin release averaging 89.5 ± 2.6 per cent (n = 99; pooled data obtained with all drugs) of the mean control value. At a 10^{-6} M concentration, all drugs completely suppressed glucose-induced insulin release, the overall mean value averaging −1.3 ± 3.3 per cent (n = 99) of the mean control rate of secretion. In between these two concentrations, a dose-related inhibition of insulin release was observed (Fig. 1).

The slope of the regression line characterizing the inhibitory effect of each drug upon glucose-stimulated insulin release was almost the same in all cases, each ten-fold increase in drug concentration causing a 41.1 ± 1.2 per cent fall in insulin output (Table 2). However, the elevation of the regression line were not identical. As judged from the ED_{50}, the potency of the drugs to inhibit insulin release ranged as follows: Bay a 1040 < Bay e 5009 < Bay k 9320 ~ Bay e 9736 ~ Bay k 5552 (Fig. 1). There was almost a ten-fold difference in ED_{50} between the weakest and most potent drug.

The relative potency of each drug to inhibit insulin release, as characterized by the dose–action relationships (Fig. 1), was confirmed by comparing, within the same experiments, the effect of a fixed concentration ($7 \cdot 10^{-8}$ M) of each drug (Table 2). The latter comparison again yielded the following order of potency: Bay a 1040 < Bay e 5009 < Bay k 9320 ~ Bay k 5552 ~ Bay e 9736.

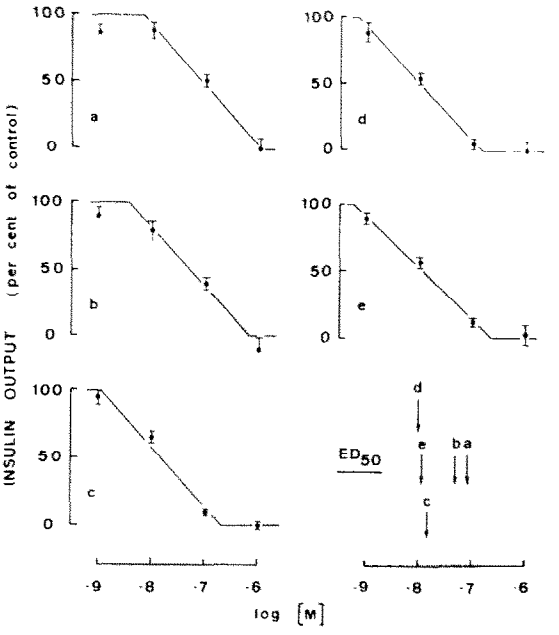

Fig. 1. Dose–action relationship for the effect of five drugs upon glucose-stimulated insulin release. Mean values (± S.E.M.) for insulin output are expressed in per cent of the mean control value found within the same experiment(s), and refer to 18–27 individual observations in each case. The symbols (a) to (e) are the same as those used in Table 2. The ED_{50} for each drug was calculated from the equation of the regression line and is shown in the lower right panel.

DISCUSSION

The results obtained in the present study with Bay a 1040 (nifedipine) are in good agreement with those

Table 2. Comparison of the effects of 5 drugs upon insulin release*

Line	Drug	Insulin output at $7 \cdot 10^{-8}$ M (% of control)	Regression analysis			
			Range (M)	ED_{50}(M)	Slope (%/log D)	r
(a)	Bay a 1040	48.7 ± 3.9 (42)	10^{-8}–10^{-6}	8.4×10^{-8}	44.1	0.9961
(b)	Bay e 5009	35.0 ± 3.4 (34)	10^{-8}–10^{-6}	4.9×10^{-8}	43.8	0.9968
(c)	Bay k 9320	14.1 ± 2.9 (34)	10^{-9}–10^{-7}	1.4×10^{-8}	42.8	0.9847
(d)	Bay k 5552	11.7 ± 4.4 (34)	10^{-9}–10^{-7}	1.0×10^{-8}	41.2	0.9950
(e)	Bay e 9736	9.9 ± 2.3 (34)	10^{-9}–10^{-7}	1.2×10^{-8}	38.4	0.9956

* The symbols (a) to (e) refer to the data illustrated in Fig 1. The mean value (± S.E.M) for insulin output evoked by glucose (16.7 mM) in the presence of each drug ($7 \cdot 10^{-8}$ M) is expressed as per cent of the mean control value found within the same experiment(s) in the absence of the drug, and is shown together with the number of individual observations (in parentheses). The regression analysis of the mean data shown in Fig. 1, as performed over the stated range of drug concentrations, is characterized by the ED_{50}, the slope of the regression line (per cent fall in insulin output for each ten-fold increase in drug concentration), and the coefficient of correlation (r) between mean values for insulin output and drug concentration.

reported in a previous study [11], in which the drug was found to cause a dose-related inhibition of insulin release evoked by glucose (16.7 mM) in isolated rat islets. In this previous study, the ED_{50} was close to $0.8 \cdot 10^{-7}$ M and the fractional inhibition amounted to approximately 36 per cent for each ten-fold increase in drug concentration. We have previously shown that the effect of nifedipine upon islet function was compatible with a primary site of action on the level of the the transport system mediating the entry of calcium into islet cells.

The present study aimed at comparing the effect of nifedipine to that of four chemically related drugs. The close similarity in the slope of the regression line characterizing the inhibitory effect of each drug upon glucose-stimulated insulin release (Fig. 1 and Table 2) is compatible with the idea that all the present drugs share a common mode of action in the endocrine pancreas. As a matter of fact, the inhibitory action of all organic calcium-antagonists so far examined in the present system is characterized by dose–action relationships with comparable slopes, despite wide variations in their respective ED_{50} [11].

In this study, the ED_{50} of the most potent drugs was almost one order of magnitude lower than that of the weakest agent. Comparison of the drugs' structure (Table 1) and dose–action relationship (Table 2) suggests that elongation of the alkyl chain in the esters of pyridine-3,5-dicarboxylic acid was associated with increased biological potency. The latter may also be affected by the nature and position of the substitution in the phenyl ring. The mechanisms which regulate the biological potency of the different drugs, as a function of their structure, were not investigated.

The present results indicate that it is possible, with our *in vitro* system, to obtain a reliable estimation of the relative potency of different organic calcium-antagonists to inhibit glucose-stimulated insulin release, and suggest that such an antagonistic action is not an uncommon property of the dihydrodimethyl-phenyl-pyridine derivatives.

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