A further examination of the possible effects of indapamide on glucose tolerance and insulin secretion in the rat and mouse

BRIAN L. FURMAN*, TARIQ BIN ABDUL RAZAK†, *Department of Physiology and Pharmacology, University of Strathclyde, George Street, Glasgow, G1 1XW, Scotland, U.K.*

The benzothiadiazine and other diuretics may produce hyperglycaemia and impaired glucose tolerance in hypertensive patients (Lewis et al 1976; Amery et al 1978). Although animal experiments demonstrate clearly the hyperglycaemic effect of the non-diuretic, antihypertensive benzothiadiazine drug diazoxide, there is no firm evidence that diuretics produce in animals an hyperglycaemic response that can be related to the effect seen in man (Furman 1977). It is nevertheless necessary to examine new antihypertensive diuretic drugs for diazoxide-like metabolic actions in experimental animals. Indapamide is a recently marketed drug with both diuretic and antihypertensive properties (Moore et al 1977). The drug is without effect on blood glucose, glucose tolerance or glucose-induced insulin secretion when given acutely to mice or rats (Furman 1977). However, there is evidence that prolonged treatment is required in man before the effects of other diuretics on glucose tolerance become manifest (Lewis et al 1976; Amery et al 1978). Therefore the effects of indapamide on glucose tolerance and glucose-induced elevations in plasma insulin have been examined after 24 day oral administration to rats and mice. Moreover, indapamide has been reported to inhibit inward calcium currents in vascular smooth muscle, an effect which could explain the depressant effect of the drug on vascular contractility in vitro and thus, possibly, its antihypertensive effect (Gargouil & Mironneau 1977). If indapamide inhibited inward calcium movements in the insulin secreting cells an inhibition of glucose-induced insulin release would be anticipated (Lorenz et al 1979). For this reason an investigation has been carried out of the effects of indapamide on basal and glucose-induced insulin release using the isolated, perfused pancreas of the rat.

Method

Rats or mice were dosed orally with indapamide (10 mg kg^{-1} daily for 24 days) as a suspension or solution respectively. Glucose tolerance procedures, carried out on the 25th day after an overnight fast, and biochemical determinations were performed as described previously (Furman 1972, 1977). In vitro insulin release was studied in the rat pancreas, isolated and perfused according to the method of Loubatières et al (1972). Indapamide or diazoxide were added directly to the perfusing medium.

Results were expressed as mean values ± s.e.m. Statistical significance was determined in the in vivo experiments using Student's t-test for unpaired observations and in the

* Correspondence.
† Present address: Department of Pharmacology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur.

in vitro experiments using the non-parametric Mann-Whitney (Wilcoxon) U test. Differences were considered to be statistically significant where $P < 0.05$.

The drugs used were indapamide (a gift from Servier Laboratories) and diazoxide (a gift from Allen and Hanbury's).

Results

The daily administration of indapamide to mice produced no effect on fasting plasma glucose concentration or the plasma concentration of glucose at 60 or 120 min after a glucose load. However, at 30 min after the glucose load there was a significantly higher glucose concentration in indapamide-treated compared with control mice, Table 1 ($P < 0.025$). Fasting plasma glucose concentrations in both groups were considerably higher than those normally seen in these mice in our laboratory, perhaps due to stress produced by daily oral injections.

The similar administration of indapamide to rats did not modify intravenous glucose tolerance or the elevation in plasma immunoreactive insulin (IRI) produced by glucose injection. Disappearance constants for glucose in the control and indapamide-treated rats were, respectively, 5.3 ± 0.5 (n = 7) and 5.93 ± 0.5 (n = 5) ($P > 0.05$). The peak plasma IRI concentration (5 min after glucose injection) in treated rats was 64 ± 15 μU ml^{-1} (fasting value 18 ± 3.5 μU ml^{-1}) compared with 58 ± 7 μU ml^{-1} in controls (fasting value 15 ± 4 μU ml^{-1}). These differences
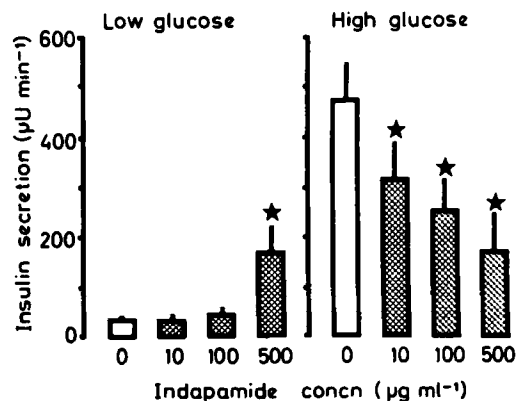

FIG. 1. Effect of various concentrations of indapamide on basal (glucose concentration − 0.6 mg ml^{-1}) and stimulated (glucose concentration − 3 mg ml^{-1}) insulin secretion by the isolated perfused pancreas of the rat. The results are expressed as the average insulin secretion per min over the time period of perfusion with either low or high concentrations of glucose. Each value is the mean ± s.e.m. of the results obtained using 5 preparations.

Table 1. The effect of 24 day treatment with indapamide (10 mg kg^{-1} day^{-1} p.o.) or vehicle on oral glucose tolerance in mice. Each value is the mean ± s.e.m.

| | Plasma glucose (mg dl^{-1}) Time (min) after oral glucose | | | |
	0	30	60	120
Control (n = 8)	160 ± 10	207 ± 16	204 ± 7	162 ± 5
Indapamide (n= 8)	152 ± 12	275 ± 20*	210 ± 20	151 ± 8

* $P < 0.025$ vs control.

were not significant and no differences were found at 10, 15 or 20 min after glucose injection.

In the isolated perfused pancreas of the rats indapamide in a concentration of 0·1 or 1·0 μg ml^{-1} did not modify basal or glucose-induced insulin release. Higher concentrations (10 or 100 μg ml^{-1}) produced a dose-dependent and statistically significant reduction in the total insulin secretory response to the high concentration of glucose during a 20 min perfusion period. In a concentration of 500 μg ml^{-1} indapamide completely suppressed the stimulatory effect of the high glucose concentration. In this concentration indapamide also produced a significant increase in the basal insulin secretion rate ($P < 0.05$) (Fig. 1).

Diazoxide, in a concentration of 50 μg ml^{-1}, prevented completely the stimulatory effect of a high concentration of glucose without modifying basal secretion. Basal (control) 42 ± 7; high glucose (control) 478 ± 68; Basal (diazoxide) 57 ± 6; high glucose (diazoxide) 52 ± 6 μU IRI pancreas^{-1} min^{-1}; high glucose (diazoxide) vs high glucose (control) − $P < 0.01$.

Discussion

The results obtained in the experiments in which rats and mice were dosed orally for 24 days appear to confirm, in general, the impression obtained from the acute experiments (Furman 1977) that indapamide is without effect on glucose tolerance or glucose-induced release in the rat or mouse. Extrapolation of these findings to the clinical situation must, however, be cautious in view of the failure of similar experiments to demonstrate any effect of hydrochlorothiazide (Foy & Furman 1972), which has been found to impair glucose tolerance in man (Amery et al 1978). The early elevation of the blood glucose concentration at 30 min after the oral glucose load in indapamide-treated mice is similar to that reported after 14 day administration of ethacrynic acid or frusemide (Foy & Furman 1972). The explanation for this is unknown but may be related to a reduced volume of distribution due to a diuretic-induced reduction in the extracellular fluid volume, or to some stimulation of glucose absorption produced by the drug.

Inhibition of glucose-induced insulin release in the isolated perfused pancreas of the rat occurred only at high concentrations of indapamide. However these concentrations are in the same range as those reported to have direct relaxant effects on vascular smooth muscle and to depress inward calcium currents in such tissue (Moore et al 1977;

Gargouil & Mironneau 1977). The steady-state plasma indapamide concentration after the administration of a therapeutic dose of the drug (2·5 mg) has been found to average about 30 ng ml^{-1} and the peak plasma concentration after a large dose (10 mg) was reported to be 140 ± 50 ng ml^{-1} (Campbell et al 1977). There is thus a marked discrepancy between the 'therapeutic' concentration range and the concentration range producing effects on vascular smooth muscle or on the pancreas. If the demonstrated direct relaxant effect of indapamide on vascular smooth muscle is indicative of the mechanism of its antihypertensive action, then the sensitivity of vascular smooth muscle in vivo must be greater than that seen in vitro. Moreover, in view of the apparent absence of effects of indapamide on insulin secretion in vivo there must be a marked separation of the in vivo sensitivities of vascular smooth muscle and the β cells of the pancreatic islets to the actions of the drug. It is possible that the isolated perfused pancreas preparation from the rat is insensitive to the drug compared with the same tissue in vivo in man. However, this seems unlikely in view of the complete inhibition of glucose-induced insulin secretion produced in this preparation by diazoxide, a drug known to inhibit insulin secretion in man, in a concentration compatible with that found in the plasma after the oral administration of the drug (Calesnick et al 1965; Sadee et al 1973). The mechanism of the demonstrated inhibitory effect of indapamide on insulin secretion remains to be determined. However it may be speculated that the effect is analogous to the relaxant effect of indapamide on vascular smooth muscle, an action suggested to be mediated by an inhibition of inward calcium currents (Gargouil & Mironneau 1977). In this respect the compound, in large doses, may behave like diazoxide in its effects on vascular smooth muscle (Janis & Triggle 1973) and the insulin secreting cells (Malaisse et al 1973).

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Inhibition of histamine-induced acid secretion in rat isolated gastric mucosa by esters of phorbol and 12-deoxyphorbol

JACALYN B. PEARCE, A. W. BAIRD, ELIZABETH M. WILLIAMSON, F. J. EVANS*, *Departments of Pharmacology and Pharmacognosy*, The School of Pharmacy, University of London, 29–39, Brunswick Square, London, WC1N 1AX, U.K.*

Tigliane esters, isolated from plants of the family Euphorbiaceae (Evans & Soper 1978) are biologically active and have been shown to be potent skin irritants (Schmidt & Evans 1980) and to cause a two-stage aggregation of human platelets (Westwick et al 1981). In this communication we describe the antisecretory activity of four tigliane derivatives on the rat isolated gastric mucosa (Main & Pearce 1978).

Method

Immature rats of either sex (80 to 100 g, home bred from the Olac strain), allowed free access to food and water, were anaesthetized with pentobarbitone (60 mg kg^{-1} s.c.) and the stomach exteriorized. The muscle layer overlying the non-antral glandular region was separated from the mucosa by blistering (Main & Pearce 1978). The muscle sheet was pulled back to expose the mucosa; the stomach was then removed and the rat killed. Two 1 cm^2 pieces of mucosa from the stomach were placed in organ baths containing 35 ml of a buffered Krebs' solution at 37 °C (serosal solution [mM]; NaCl 110·0, KCl 5·0, CaCl$_2$.6H$_2$O 3·6, MgCl$_2$.6H$_2$O 1·2, Na HCO$_3$ 26·0, glucose 16·7), gassed with a 95% O$_2$ and 5% CO$_2$ mixture, which bathed the serosal surface of the preparation. The mucosal surface was superfused at 0·5 ml min^{-1} with an unbuffered solution (mucosal solution [mM]; NaCl 136·0, KCl 5·0, CaCl$_2$.6H$_2$O 3·6, MgCl$_2$.6H$_2$O 1·2, glucose 16·7) gassed with O$_2$. Acid output was recorded via a dual micro-electrode in the mucosal solution, connected to an antilog unit and a potentiometric pen recorder.

Experimental design. Paired mucosae, from a single stomach, were allocated to treatment groups. The secretagogue, histamine or pentagastrin, was added to all mucosae 120 min into the experiment, and two further responses were obtained at 210 and 300 min. For each response, the secretagogue was added to the serosal solution and left in contact for 30 min; consecutive responses were separated by a 60 min washout period. One mucosa from each pair, the untreated control, was used to monitor time-dependent changes in response to the secretagogue. A test compound was added to the second tissue at 180 min, 30 min before the second response, and left in contact for 60 min.

* Correspondence.

The acid secretory response is calculated as the increase in output at the peak of the response (P) over the preceding basal rate (B; i.e. P–B). The effect of the test compound was assessed by comparing the magnitude of the response in the treated mucosa with that in the paired control. Data were analysed by the Wilcoxon matched Pairs test (1 tailed; $P \leqslant 0.05$ was considered to be significant).

Drugs. Solutions of pentobarbitone (Nembutal, Abbott), histamine acid phosphate (BDH) and pentagastrin (Peptavlon, ICI) were prepared in 0·15 M NaCl solution. Phorbol, isolated from the seed oil of *Croton tiglium,* was dissolved in ethanol. 12-Deoxyphorbol phenylacetate (12-DOPPA) and 12-deoxyphorbol phenylacetate-20-acetate (12-DOPPAA), isolated from the latex of *Euphorbia Poisonii Pax* (Schmidt & Evans 1979), were dissolved in acetone, as was 12-*O*-tetradecanoyl phorbol acetate (TPA, Sigma).

Results

In mucosae treated with phorbol (4×10^{-6} M, n = 6, first histogram in Fig. 2), the response to histamine (5×10^{-5} M) was slightly, but not significantly, reduced in the presence of the test compound; the third response was similar to that initially obtained in these mucosae. The same pattern of responses was observed in the untreated control preparations; histamine-induced secretion was reduced at the second response but subsequently increased. Between paired test and control mucosae, there were no significant differences in the secretory responses to histamine.

As shown in the second histogram, TPA (10^{-7} M, n = 11) produced a small but significant inhibition of histamine-induced secretion. In preparations treated with TPA consecutive responses to histamine were similar in magnitude. However, the responses in control mucosae increased steadily throughout the experiment. There was a significant reduction in secretion, relative to the controls, in the presence of TPA ($P < 0.001$) and in the response following its removal ($P < 0.001$).

Similarly, in experiments with 12-DOPPA (3×10^{-7} M, n = 6, third histogram) responses to histamine were significantly reduced in the presence of the ester ($P < 0.05$) and at the subsequent response ($P < 0.05$), following its removal. Inhibition of histamine-induced secretion by 12-DOPPAA (3×10^{-7} M, n = 6, fourth histogram) was more marked. Although the response in the presence of