*J. Pharm. Pharmac.*, 1974, **26**, 512–517 *Received November* 29, 1973

# Effect of some β-adrenoceptor blocking drugs on insulin secretion in the rat

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In pentobarbitone anaesthetized rats (±)-propranolol reduced the stimulatory effect of glucose, sulphonylureas, isoprenaline or phentolamine on insulin secretion. (+)-Propranolol produced similar effects to those produced by the racemate. Sotalol reduced only the insulin secretion stimulated by isoprenaline or phentolamine but not that stimulated by glucose or sulphonylureas. Complete inhibition of isoprenaline-induced hyperinsulinaemia was obtained with practolol, in a dose which was without effect on the plasma insulin elevations produced by glucose or glibenclamide. (±)-Propranolol inhibited glucose or tolbutamide-stimulated insulin secretion from chopped pancreas *in vitro* indicating a direct action of the drug on the pancreas. It is suggested that propranolol-induced inhibition of insulin secretion may not be entirely due to the β-adrenoceptor blocking activity of the drug.

Propranolol has been reported to inhibit glucose- and/or sulphonylurea-stimulated insulin secretion in man, dog and mouse (Bressler, Vargas-Cardon & Brendel, 1969; Sirek, Vigas & others, 1969; Majid, Saxton & others, 1970; Cerasi, Luft & Efendic, 1972). These observations have been interpreted as further evidence for a role of the sympathetic nervous system in the physiological regulation of insulin secretion or as evidence that both glucose and sulphonylureas increase insulin secretion by mechanisms involving the activation of β-adrenoceptors in the islets of Langerhans. On the other hand, Bressler & others (1969) questioned the specificity of propranolol as a β-adrenoceptor blocking agent in such experiments. We have attempted to obtain further information concerning the action of propranolol on insulin secretion using the rat and have compared the drug's effects with those of two other β-adrenoceptor blocking drugs, sotalol and practolol. Some of these results were presented to the British Pharmacological Society and have been published in abstract form (Furman & Tayo, 1973).

## METHODS

### *In vivo studies*

Male Wistar rats (200–300 g) deprived of food overnight were anaesthetized with pentobarbitone sodium (60 mg $kg^{-1}$, i.p.). The femoral artery and vein of one leg were cannulated for blood sampling and drug administration respectively. The animals were heparinized (100 U, i.v.). Arterial blood samples (0·2 ml) were collected after discarding 0·2 ml to allow for the dead-space in the cannula. The blood was immediately centrifuged and 25 μl of plasma was transferred to a microtest tube and stored deep frozen for subsequent insulin determination. All drugs were injected intravenously in a volume of 0·1 ml and washed in with 0·2 ml 0·9% sodium chloride solution.

*In vitro studies*

The splenic portion of the pancreas was removed from fasted anaesthetized rats, chopped with scissors and incubated at 37° in Krebs-Ringer bicarbonate buffer containing 2 mg $ml^{-1}$ bovine albumin and gassed with 5% $CO_2$ in oxygen (Coore & Randle, 1964). Three or four pieces of pancreas were incubated for 15 min in 3 ml low glucose buffer (0·6 mg $ml^{-1}$) and 25 $\mu$l of the medium was frozen for subsequent insulin determination. The tissue was then washed and incubated for a second 15 min period in 3 ml of buffer containing a high concentration of glucose (3 mg $ml^{-1}$) or a low concentration of glucose (0·6 mg $ml^{-1}$) together with tolbutamide (200 $\mu$g $ml^{-1}$). These incubations were carried out in the presence or absence of propranolol (0·5 $\mu$g $ml^{-1}$). The results are expressed in terms of the difference ($\Delta$ insulin, $\mu$U $ml^{-1}$) between the insulin concentrations present at the end of the two incubations.

*Determination of plasma glucose.* Glucose was determined in 10 $\mu$l plasma using a glucose oxidase method (Beckman Glucose Analyser).

*Determination of immunoreactive insulin (IRI) in plasma or incubation media.* IRI was determined using the double antibody radio-immunoassay procedure of Hales & Randle (1963) as modified by the Radiochemical Centre, Amersham. Plasma or buffer was diluted 1 in 2 before the determination. A separate standard curve was plotted for each batch to be assayed, using human insulin in protein buffer solution in concentrations from 0–200 $\mu$U $ml^{-1}$. Samples were determined in duplicate.

*Experimental design.* In all experiments appropriate control animals were examined under the same conditions and at the same time as animals receiving the drug being examined. Results are expressed in terms of a human insulin standard.

*Drugs used.* These were: (±)-, (+)-propranolol hydrochloride and practolol (ICI); glibenclamide and tolbutamide (Hoechst); (−)-isoprenaline bitartrate (Wyeth); phentolamine mesylate (CIBA); sotalol hydrochloride (Mead Johnson). All drugs were dissolved in 0·9% sodium chloride solution. Glibenclamide and tolbutamide were rendered soluble using drops of N sodium hydroxide solution. All doses refer to the active molecule. Statistical significance was determined using Student's *t*-test or analysis of covariance where appropriate, significance being accepted where $P<0·05$.

## RESULTS

*Fasting plasma glucose and insulin concentrations.* Propranolol (0·5 mg $kg^{-1}$) had no consistent effect on fasting plasma glucose and insulin concentrations. In some experiments, however, a significant reduction in these values was found in propranolol treated rats (Table 1).

*Glucose-stimulated insulin secretion.* Glucose (0·5 g $kg^{-1}$) (2 ml $kg^{-1}$ of a 25% solution injected i.v. over 60 s) produced a prompt rise in both plasma glucose and insulin concentrations. (±)-Propranolol (0·5 mg $kg^{-1}$ injected 15 min before glucose) markedly reduced the plasma insulin elevation produced by glucose injection. Surprisingly, glucose-induced hyperglycaemia was also reduced (Table 1).

Table 1. *The effect of (±)-propranolol (0·5 mg kg$^{-1}$) on the plasma glucose and plasma immunoreactive insulin (IRI) responses to glucose, glibenclamide, isoprenaline or phentolamine.* Although individual control values showed differences between different days, control solution administration did not produce changes in plasma glucose or IRI concentrations relative to fasting levels. Individual control experiments are not shown. Propranolol was injected 15 min before the drug under test. *indicates the value to be significantly different from the value obtained in the absence of propranolol. †indicates the value to be significantly different from the fasting value ($P<0·05$).

| | | Plasma glucose (mg dl$^{-1}$) | | | Plasma IRI (μU ml$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| Treatment | No. of observations | Fasting | 5 min post drug | 20 min post drug | Fasting | 5 min post drug | 20 min post drug |
| Control | 5 | 97 ± 8 | 104 ± 6 | 103 ± 8 | 25 ± 6 | 28 ± 2 | 20 ± 3 |
| Propranolol | 5 | 98 ± 2 | 105 ± 5 | 105 ± 5 | 17 ± 5 | 28 ± 5 | 22 ± 6 |
| Glucose 0·5 g kg$^{-1}$ | 8 | 126 ± 8 | 250 ± 8† | 148 ± 10 | 50 ± 3 | 173 ± 8† | 98 ± 9† |
| Glucose + propranolol | 8 | 99 ± 13 | 181 ± 10†* | 71 ± 6* | 49 ± 6 | 72 ± 6†* | 71 ± 8† |
| Glibenclamide 2 mg kg$^{-1}$ | 7 | 109 ± 3 | 86 ± 6† | 59 ± 3† | 80 ± 8 | 205 ± 9† | 213 ± 11† |
| Glibenclamide + propranolol | 7 | 95 ± 5 | 117 ± 5† | 77 ± 5† | 58 ± 10* | 133 ± 8†* | 139 ± 8†* |
| Isoprenaline 175 μg kg$^{-1}$ | 7 | 105 ± 8 | 138 ± 5† | 184 ± 10† | 54 ± 3 | 174 ± 8† | 320 ± 15† |
| Isoprenaline + propranolol | 7 | 82 ± 3* | 73 ± 5* | 64 ± 8†* | 29 ± 10* | 49 ± 6†* | 65 ± 5†* |
| Phentolamine 5 mg kg$^{-1}$ | 6 | 134 ± 8 | 157 ± 5† | 138 ± 10 | 30 ± 6 | 79 ± 8† | 55 ± 10† |
| Phentolamine + propranolol | 6 | 129 ± 3 | 151 ± 6† | 131 ± 11 | 32 ± 8 | 61 ± 5†* | 37 ± 8* |

*Sulphonylurea-stimulated insulin secretion.* Glibenclamide (2 mg kg$^{-1}$, i.v.) produced a marked elevation in plasma IRI concentrations, accompanied by hypoglycaemia. The hyperinsulinaemia was significantly reduced by (±)-propranolol (administered as previously) and the hypoglycaemic response was reduced (Table 1). Experiments in which the time course of the glibenclamide hypoglycaemia has been followed further have shown that, despite the early inhibition of the response by propranolol, plasma glucose levels are eventually reduced to the same extent as that seen in animals which have not received the blocking drug. (±)-Propranolol similarly reduced the plasma insulin increase produced by tolbutamide (200 mg kg$^{-1}$ i.v.).

*Isoprenaline-induced secretion.* Administration of isoprenaline (175 μg kg$^{-1}$ injected over 3 min) produced hyperglycaemia and an increase in the plasma IRI concentration. Although these effects were always obtainable both responses showed considerable quantitative variations when the experiments were repeated on different days. (±)-Propranolol (administered as previously) virtually abolished both responses (Table 1).

*Phentolamine-induced insulin secretion.* Phentolamine treatment (5 mg kg$^{-1}$, i.v.) resulted in a small hyperglycaemic response and a marked elevation in plasma IRI concentrations. The hyperinsulinaemia, but not the hyperglycaemia was significantly reduced by (−)-propranolol administered 15 min before phentolamine (Table 1).

*Effect of (+)-propranolol on the plasma insulin changes produced by glucose sulphonylureas and isoprenaline.* (+)-Propranolol (0·25 mg kg$^{-1}$) modified the plasma insulin responses to glucose, sulphonylureas and isoprenaline in a manner qualitatively similar to the racemic mixture.

Table 2. *The effect of sotalol (50 mg kg$^{-1}$) on the plasma glucose and immunoreactive insulin (IRI) responses to glucose, glibenclamide, isoprenaline or phentolamine.* Sotalol was injected 15 min before the drug under study. *Indicates the value to be statistically significantly different from the appropriate value obtained in the absence of sotalol. †Indicates value to be significantly different from fasting value ($P<0.05$).

| | | Plasma glucose (mg dl$^{-1}$) | | | Plasma IRI ($\mu$U ml$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| Treatment | No. of observations | Fasting | 5 min post drug | 20 min post drug | Fasting | 5 min post drug | 20 min post drug |
| Control | 5 | 106 ± 8 | 117 ± 10 | 110 ± 8 | 23 ± 6 | 23 ± 8 | 25 ± 6 |
| Sotalol | 5 | 109 ± 5 | 129 ± 5 | 124 ± 5 | 19 ± 7 | 20 ± 5 | 21 ± 5 |
| Glucose 0·5 g kg$^{-1}$ | 6 | 121 ± 5 | 341 ± 15 | 177 ± 6 | 34 ± 12 | 103 ± 12 | 40 ± 6 |
| Glucose + sotalol | 6 | 138 ± 3 | 325 ± 20 | 226 ± 14 | 28 ± 4 | 75 ± 10 | 43 ± 5 |
| Glibenclamide 2 mg kg$^{-1}$ | 6 | 105 ± 5 | 113 ± 8 | 79 ± 4 | 26 ± 3 | 65 ± 5 | 76 ± 2 |
| Glibenclamide + sotalol | 6 | 117 ± 4 | 105 ± 2 | 83 ± 3 | 20 ± 3 | 64 ± 6 | 66 ± 4 |
| Isoprenaline 175 $\mu$g kg$^{-1}$ | 5 | 102 ± 4 | 117 ± 2† | 133 ± 2† | 31 ± 2 | 55 ± 2† | 65 ± 2† |
| Isoprenaline + sotalol | 5 | 104 ± 4 | 119 ± 1† | 134 ± 2 | 24 ± 4 | 37 ± 4†* | 48 ± 4†* |
| Phentolamine 5 mg kg$^{-1}$ | 7 | 112 ± 5 | 136 ± 6 | 109 ± 7 | 10 ± 4 | 35 ± 7† | 24 ± 3† |
| Phentolamine + sotalol | 7 | 121 ± 5 | 94 ± 7 | 94 ± 7 | 12 ± 1 | 14 ± 1* | 26 ± 5† |

*Effect of sotalol on plasma insulin concentrations.* Sotalol, in doses between 5 and 50 mg kg$^{-1}$, did not significantly modify the plasma insulin response to glucose or glibenclamide. However a dose of 50 mg kg$^{-1}$ significantly reduced the increase in plasma insulin produced by isoprenaline or phentolamine (Table 2).

*Effect of practolol on plasma insulin concentrations.* Practolol (8 mg kg$^{-1}$) completely prevented the increased immunoreactive insulin levels produced by isoprenaline but did not modify the responses to glucose or glibenclamide (Fig. 1).

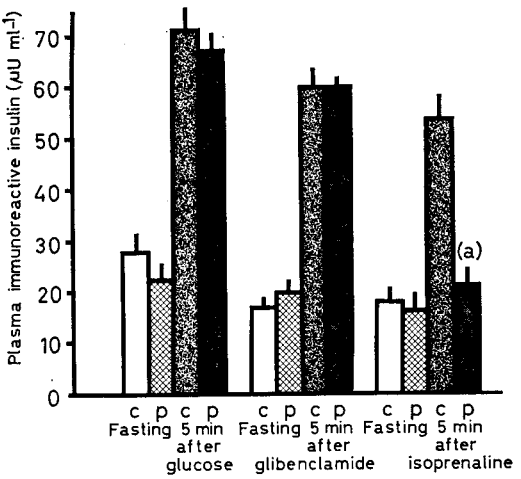


FIG. 1. The effect of practolol (8 mg kg$^{-1}$, i.v.) on plasma immunoreactive insulin values in the fasting state and 5 min after the injection of glucose (0·5 g kg$^{-1}$), glibenclamide (2 mg kg$^{-1}$) or isoprenaline (175 $\mu$g kg$^{-1}$). Practolol was given 15 min before the drug under test. Each column represents the mean of six observations. The vertical bars indicate the standard error of the mean.
(a) indicates a statistically significant difference between values obtained in rats receiving practolol (p) and rats receiving the appropriate control vehicle (c) ($P<0.05$).
Values at other time intervals are not shown but practolol produces the same effect (or lack of effect) on the responses to the drugs under test, at other time intervals, as shown above.

*Propranolol on insulin secretion in vitro.* Glucose (3 mg ml$^{-1}$) or tolbutamide (200 $\mu$g ml$^{-1}$) significantly increased the secretion of insulin by pieces of rat pancreas incubated *in vitro*. Although (±)-propranolol (0·5 $\mu$g ml$^{-1}$) did not affect basal insulin secretion it markedly reduced the stimulatory effect of glucose and abolished that of tolbutamide (Fig. 2).

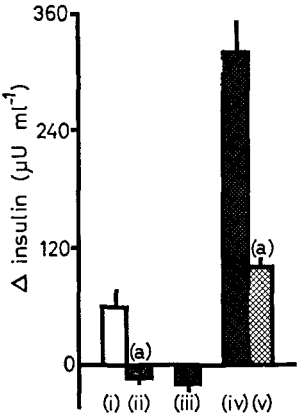


FIG. 2. The effect of propranolol (0·5$\mu$g ml$^{-1}$) on the secretion of insulin from pieces of chopped pancreas. The results are expressed in terms of Δ insulin ($\mu$U ml$^{-1}$), which is the difference found in insulin concentrations between two successive incubations. The first incubation was carried out in a low glucose (0·6 mg ml$^{-1}$) medium without drug. The second incubation was carried out in a medium containing either (i) glucose 0·6 mg ml$^{-1}$ + tolbutamide 200 $\mu$g ml$^{-1}$ or (ii) glucose 0·6 mg ml$^{-1}$ + tolbutamide 200 $\mu$g ml$^{-1}$ + propranolol or (iii) glucose 0·6 mg ml$^{-1}$ + propranolol or (iv) glucose 3 mg ml$^{-1}$ or (v) glucose 3 mg ml$^{-1}$ + propranol.
Each column represents the mean of 7-9 observations. The vertical bars indicate the standard errors. (a) indicates the value to be significantly different from the appropriate control value ($P < 0.05$).

*Effect of propranolol on the immunoassay procedure.* In view of the marked and unspecific effect of propranolol on the plasma insulin responses to various agents it was especially important to examine the possibility that the drug interfered with the immunoassay of insulin. A series of human insulin standards was prepared (0–80 $\mu$U ml$^{-1}$) containing 0, 5, 10 or 20 $\mu$g ml$^{-1}$ (±)-propranolol and the samples were assayed for insulin. No significant difference was found between the estimated insulin concentrations in samples assayed in the presence and absence of propranolol. Furthermore, propranolol (5 $\mu$g ml$^{-1}$) did not influence the determination of insulin in pooled rat plasma.

## DISCUSSION

Our results show that propranolol can inhibit insulin secretion in the rat as previously shown in other species. Inhibition of insulin secretion by propranolol *in vitro* suggests that the effect is exerted directly on the islets of Langerhans and is not mediated through primary effects of the drug on blood glucose or pancreatic blood flow. Moreover, biochemical interference by propranolol with the immunoassay is unlikely to explain its effects in inhibiting insulin secretion.

The reduction of isoprenaline and phentolamine-induced increases in plasma IRI concentrations by propranolol or sotalol could be explained by the $\beta$-adrenoceptor blocking activity of the drugs. Phentolamine induced hyperinsulinaemia has been suggested to be due to unmasking by $\alpha$-adrenoceptor blockade of the $\beta$-adrenoceptor stimulatory effect of the sympathetic nervous system (Lundquist, 1972b). However,

several observations suggest that propranolol-induced inhibition of insulin secretion cannot be explained entirely in terms of β-adrenoceptor blockade. (+)-Propranolol, reported to possess very little of the β-adrenoceptor blocking action of the (−)-isomer (Barrett & Cullum, 1968), produced very similar effects to those produced by the racemic mixture. Additionally, sotalol in doses which significantly reduced the stimulatory effect of isoprenaline and phentolamine on insulin secretion did not modify the hyperinsulinaemia produced by glucose or glibenclamide. Practolol (8 mg $kg^{-1}$) completely prevented isoprenaline-induced hyperinsulinaemia without affecting the responses to glucose or sulphonylureas.

Plasma glucose changes induced by the various agents used to provoke insulin secretion were modified, in some cases, by the β-adrenoceptor drugs. However, the alteration of these responses could not be correlated with the changes in insulin secretion produced by the drugs. For example, glucose-induced hyperglycaemia was diminished by propranolol despite a marked suppressant effect of the drug on the plasma insulin concentrations. This observation may be explained by the direct stimulatory effect of propranolol on peripheral glucose utilization (Lundquist, 1972a). Phentolamine was found to produce hyperglycaemia in contrast to the findings in the rat of Senft, Sitt & others (1968). This was unexpected and is difficult to explain, especially as plasma insulin levels were markedly increased by phentolamine.

Our results suggest that propranolol can exert effects on insulin secretion which are unrelated to β-adrenoceptor blockade. This conclusion is in agreement with that of Bressler & others (1969). Moreover we have shown that it is possible to block β-adrenoceptors without altering the stimulatory effect of glucose or sulphonylureas on insulin secretion using drugs such as sotalol and practolol. It thus seems unlikely that the action of glucose and sulphonylureas on insulin secretion is mediated, to an important extent, through β-adrenoceptor stimulation. Caution is necessary in the interpretation of experiments on insulin secretion in which propranolol has been used as a tool.

*Acknowledgements*

We are grateful to Hoechst Pharmaceuticals for generous gifts of tolbutamide and glibenclamide. One of us (F.M.T.) was in receipt of a grant from the Lagos State Government of Nigeria.

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