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## ORIGINALS

# The Action of ß-Adrenergic Blocking and Stimulating Agents on Insulin Secretion. Characterization of the Type of ß Receptor.

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*Summary.* As a result of our experiments designed to study *in vivo* in the anaesthetized dog, the role of the beta-adrenergic receptors involved in insulin secretion, we have found: 1. that isoprenaline (global stimulator of the beta-adrenergic receptors) provoked a considerable increase in the secretion of insulin. — 2. that propranolol (blocking agent of the beta-adrenergic receptors) partially and temporarily inhibited the secretion of insulin. — 3. that isoprenaline, after blockage of the beta-adrenergic receptors by propranolol, provoked a strong, long-lasting inhibition of insulin secretion. — 4. that practolol (selectively $\beta_1$ blocking agent) did not counteract the stimulating effects of isoprenaline ($\beta_1$ and $\beta_2$ stimulating agent). This suggests that the beta-adrenergic receptor involved in insulin secretion is of the type $\beta_2$. — 5. that salbutamol (selective $\beta_2$ stimulating agent) provoked an abundant secretion of insulin, an effect which was found to be blocked by propranolol. This last fact confirms that the beta-adrenergic receptor involved in the insulin secretion provoked by isoprenaline is of type $\beta_2$. — All these findings underline the importance of the $\beta_2$ adrenergic receptors of the beta cell of the islets of Langerhans, in the process of insulin secretion.

*Action sur l'insulino-sécrétion des substances bloquant et stimulant les récepteurs β adrénergiques. Caractérisation du type de récepteur β*

*Résumé.* Il résulte de nos expériences destinées à étudier *in vivo*, chez le chien anesthésié, le rôle des récepteurs β-adrénergiques impliqués dans l'insulino-sécrétion: 1. que l'isoprénaline (stimulant des récepteurs bêta-adrénergiques) provoque une augmentation importante de la sécrétion d'insuline. — 2. que le propranolol (bloquant des récepteurs bêta-adrénergiques) freine temporairement la sécrétion d'insuline. — 3. que l'isoprénaline, après blocage des récepteurs bêta-adrénergiques par le propranolol, inhibe l'insulino-sécrétion d'une manière puissante et durable. — 4. que le practolol (bloquant plus sélectif des récepteurs $\beta_1$) ne s'oppose pas aux effets stimulants de l'isoprénaline qui agit sur les récepteurs $\beta_1$ et $\beta_2$, ce qui suggère que le récepteur bêta-adrénergique impliqué dans l'insulino-sécrétion est de type $\beta_2$. — 5. que le salbutamol (stimulant plus sélectif des récepteurs $\beta_2$) provoque une abondante sécrétion d'insuline, effet qui se trouve bloqué par le propranolol. Ce fait confirme que le récepteur β adrénergique impliqué dans l'insulino-sécrétion provoquée par l'isoprénaline est de type $\beta_2$. — Tous ces faits soulignent l'importance des récepteurs adrénergiques $\beta_2$ de la cellule bêta des îlots de Langerhans dans le processus d'insulino-sécrétion.

*Die Wirkung von blockierenden und stimulierenden, β-adrenergischen Substanzen auf die Insulinsekretion. Charakterisierung des β-Rezeptortyps.*

*Zusammenfassung.* Wir fanden in unseren Experimenten, die dazu angelegt waren, am anästhesierten Hund *in vivo* die Rolle der β-adrenergischen Rezeptoren für die Insulinsekretion zu erforschen: 1. daß Isoprenaline (welches die β-adrenergischen Rezeptoren stimuliert), eine starke Erhöhung der Insulinsekretion hervorruft. — 2. daß Propranolol (ein Blocker der β-adrenergischen Rezeptoren) die Insulinsekretion hemmt. — 3. daß Isoprenaline nach der Blockierung der β-adrenergischen Rezeptoren durch Propranolol die Insulinsekretion stark, und dauerhaft hemmt. — 4. daß Practolol (welches mehr die Rezeptoren $\beta_1$ hemmt) nicht die Stimulation des Isoprenaline aufhebt, welches sowohl auf die $\beta_1$ und $\beta_2$ Rezeptoren wirkt. Dieses deutet darauf hin, daß die β-adrenergischen Rezeptoren, die bei der Insulinsekretion mitwirken, dem Typ $\beta_2$ angehören. — 5. daß Salbutamol (welches mehr die $\beta_2$-Rezeptoren anregt) eine übermäßige Insulinsekretion bewirkt, die wiederum durch Propranolol zu hemmen ist. Diese Tatsache bestätigt, daß die β-adrenergischen Rezeptoren, welche an der durch Isoprenaline hervorgerufenen Insulinsekretion beteiligt sind, dem Typ $\beta_2$ angehören. — Alle diese Tatsachen unterstreichen die Bedeutung der $\beta_2$ adrenergischen Rezeptoren der β-Zelle der Langerhansschen Inseln für den Prozess der Insulinsekretion.

*Key-words:* Insulin secretion, β-adrenergic receptors, β-adrenergic blocking agents, β-adrenergic stimulating agents, isoprenaline, propranolol, practolol, salbutamol.

In this investigation we have studied the effects on insulin secretion of substances acting on the β-adrenergic receptors: some stimulating these receptors, others blocking them. These effects were observed either by administering each of the drugs separately or by administering them simultaneously.

## *Technique*

Our work was carried out on dogs anaesthetized with pentobarbital (nembutal) given intravenously at a dose of 30 mg/kg. The insulin was measured by the B-radioimmunologic method of Hales and Randle [6]

in the venous efferent blood of the pancreas. In order to do this, a T-shaped cannula was introduced between the two segments of the pancreatico-duodenal vein sectioned near its entry into the portal vein; the blood samples were drawn from the free branch of the T, and the blood flow rates were controlled. The glycaemia was measured with the Technicon Auto-Analyzer on haemolyzed blood following the method described by Alric *et al.* [1]; the blood was drawn in a continuous manner from the femoral vein. As a control measure, we simultaneously registered the arterial blood pressure.

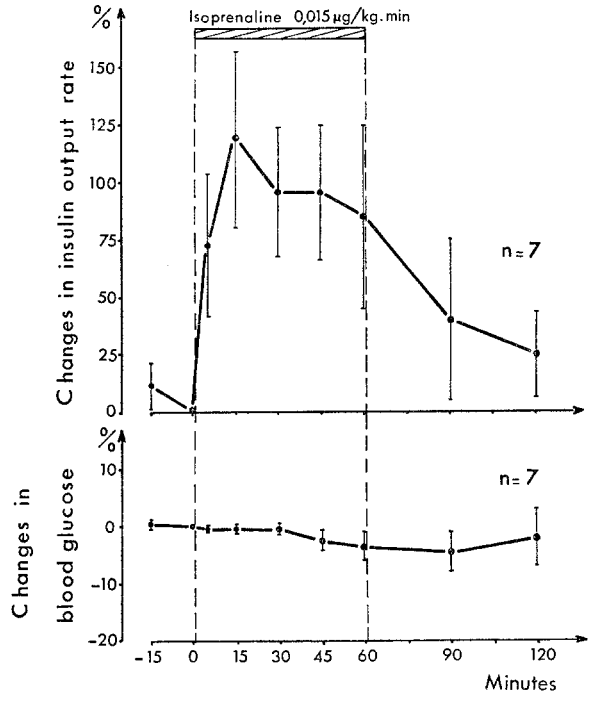


Fig. 1. Effects of DL-isoprenaline (0.015 µg/kg.min) on the insulin secretion rate in the pancreatico-duodenal vein, and on the glycaemia in the anaesthetized dog. Each point represents the mean of the values obtained in 7 dogs, with the standard error of the mean

The results shown in figure 1 enable us to note that, in these conditions, isoprenaline manifests a stimulating action on the insulin secretion: the insulin-secretory effect is immediate, persists for the 60 min that the perfusion lasts, and attenuates after its termination.

At the dosage used, this catecholamine does not provoke an increase of the glycaemia; there even appears a slight diminution that may be related to the provoked hypersecretion of insulin.

*2. The effects due uniquely to propranolol*, (blocking agent of the β-adrenergic receptors), were studied by

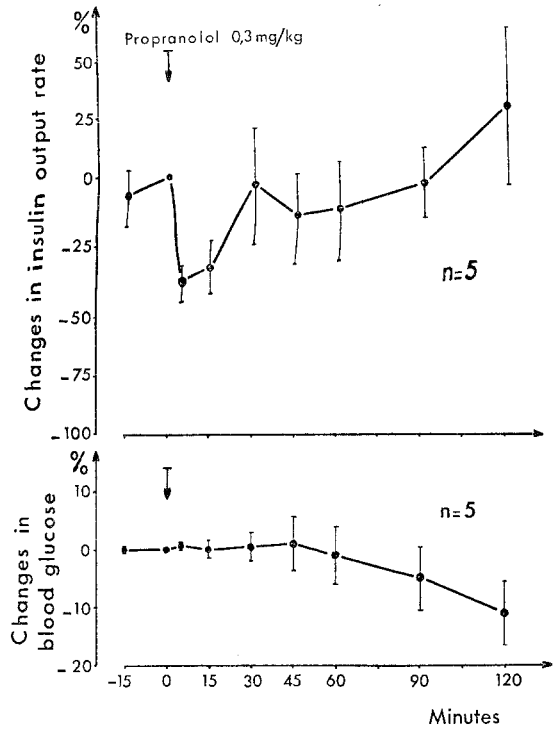


Fig. 2. Effects of propranolol administered intravenously (0.3 mg/kg) on the insulin secretion rate into the pancreaticoduodenal vein, and on the glycaemia in the anaesthetized dog. Each point represents the mean of the values obtained in 5 dogs, with the standard error of the mean

## *Results*

On the graphs that summarize our results, the changes in glycaemia and insulin-secretion rate were expressed in percentages in relation to the values registered at time zero, immediately before the administration of the drugs. As far as the insulin secretion rate is concerned, the basal secretion was, on an average, 159 ng/min; this represents 3975 µU/min, if we refer back to the equivalence of biological activity of insulin of the dog used for the preparation of the standard curve.

*1. Study of the effects of isoprenaline.* DL-isoprenaline was perfused at a dose of 0.015 µg/kg.min in the saphenous vein for 60 min; the glycaemia and insulinaemia were followed during the entire duration of the perfusion and for 60 min after its termination.

following the evolution of the secretion of insulin and of the glycaemia for 120 min after an intravenous injection of the product at a dose of 0.3 mg/kg (dose at which this substance clearly manifests a blocking action on the cardiac β-adrenergic receptors).

At this dosage (Figure 2), there is a very rapid diminution of the insulin secretion of the order of −40% that lasts less than 30 min; then the secretion evolves, on an average, between −4% and −18%, however this is not significantly different from the basal secretion level.

As for the glycaemia, it is modified in the direction of a diminution, that appears only after the 45th min; the recorded fall in glycaemia is, on the average, −11%, 120 min after the injection.

*3. The effects of the combined action of isoprenaline*

*and propranolol* are shown in Fig. 3. In these experiments, propranolol was administered by intravenous injection at a dose of 0.3 mg/kg five minutes before the beginning of the isoprenaline perfusion (0.015 μg/kg. min).

We should note that propranolol not only inhibits the stimulating action of isoprenaline on the secretion of insulin, but even reverses this action. This fact had not, up until now, been described *in vivo*, although we had already described it *in vitro* on the isolated, perfused rat pancreas [10, 8]. The reduction of the

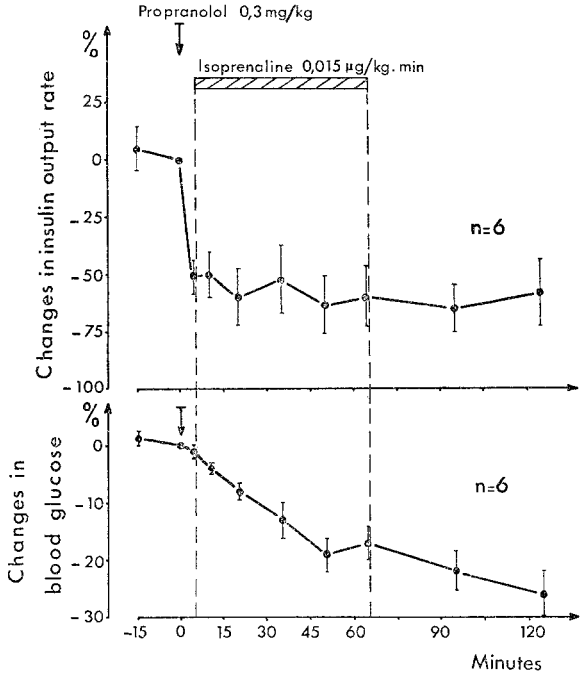


Fig. 3. Effects of DL-isoprenaline (0.015 μg/kg.min) after an intravenous injection of propranolol (0.3 mg/kg) on the insulin secretion rate into the pancreatico-duodenal vein and on the glycaemia in the anaesthetized dog. Each point represents the mean of the values obtained in 6 dogs, with the standard error of the mean

insulin secretion evolves, on the average, between −50% and −65% in a continuous manner during the 120 min that the experiment lasts; this inhibitory effect is greater and more prolonged than that noted during the administration of propranolol alone.

In the presence of propranolol, isoprenaline reveals, at the dosage used, an immediate and important hypoglycaemic action. The hypoglycaemia is at −19% at 45 min; it is prolonged and increases again after the termination of the perfusion of isoprenaline, reaching −26% at 120 min.

From the results that we have just reported, it can be seen that the activation of the β-adrenergic receptors provokes the stimulation of insulin secretion.

The next question to be considered is what type of β receptor ($\beta_1$ or $\beta_2$) was involved in this mechanism.

*4. Effects of isoprenaline in the presence of practolol.* In order to determine whether the augmentation of the insulin secretion is related to the $\beta_1$ or to the $\beta_2$ receptors, we used, instead of propranolol which blocks the two types of receptors, a substance considered as a

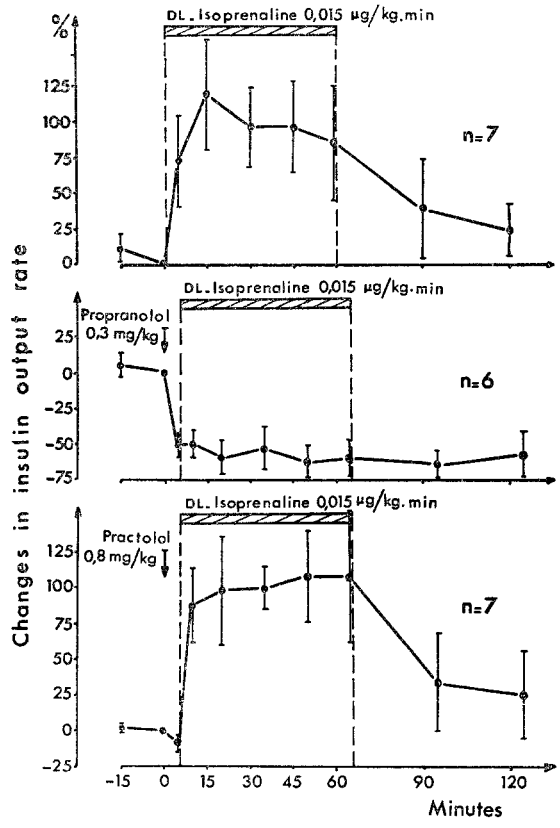


Fig. 4. Variations in the secretion rate of insulin measured in the pancreatico-duodenal vein in the anaesthetized dog, under the effect either of isoprenaline alone, or of isoprenaline in the presence of propranolol or of practolol. Each point represents the mean of the values with the standard error of the mean. The number of animals is indicated for each group of experiments

blocking agent more specifically of the $\beta_1$ receptors: ICI 50172 or practolol [4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide] (5, 2). Because of this action, this substance permits the $\beta_1$ effects to be distinguished from the $\beta_2$ effects provoked by the catecholamines.

Because practolol has, according to Barret *et al.* [2], about 40% of the activity of propranolol for opposing the effects of isoprenaline on the $\beta_1$ receptors (cardiac contractile force, cardiac rhythm and mobilization of the free fatty acids), the dose of practolol chosen was 0.8 mg/kg when that of propranolol was 0.3 mg/kg.

Like propranolol, practolol was administered by intravenous injection 5 min before the administration of isoprenaline.

In Fig. 4 are shown successively the effects on the rate of insulin secretion into the pancreatico-duodenal vein, of isoprenaline alone, of isoprenaline after administration of propranolol, and lastly of isoprenaline after administration of the $\beta_1$ blocking agent: practolol.

It was ascertained that, if propranolol suppressed and even reversed the stimulating effect of isoprenaline on insulin secretion, this stimulating effect was absolutely not modified by practolol.

*5. In a final group of experiments,* we utilized the pharmacological properties of *salbutamol,* 2-t-butylamino-1 (4-hydroxy-3-hydroxymethyl) phenylethanol, which has the property of stimulating, in a quasi-selective manner, the $\beta_2$-adrenergic receptors (4,5[bis]).

The dose of salbutamol that we used in intravenous perfusions was 0.02 μg/kg min for 60 min.

The results obtained are summarized in Fig. 5 and 6. Fig. 5 shows that salbutamol, when continuously perfused at a minimal dose in a peripheral vein (0.02 μg/kg min) strongly stimulated the secretion of insulin. This stimulation persisted for the entire duration of the perfusion (60 min) and lasted, though diminished, another 60 min period.

Fig. 6 represents a typical experiment. In the first part, is shown the effect of salbutamol on insulin secretion. In the second part, it is evident that the preliminary administration of propranolol (global blocking agent of the β receptors) in a dose of 0.3 mg/kg I.V., opposed the insulin secretory effects of salbutamol, and even reversed these effects. Therefore, these experiments strongly suggest that the β-adrenergic receptors that determine the stimulation of insulin secretion are of the $\beta_2$ type.

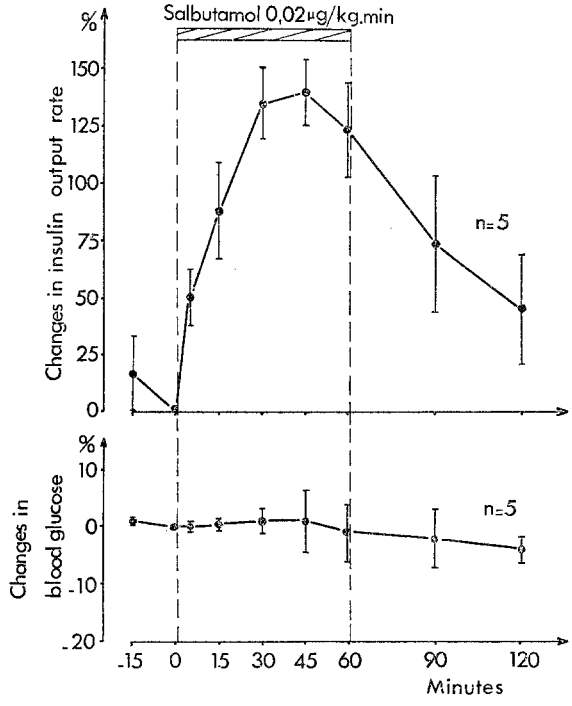


Fig. 5. Effects of salbutamol (0.02 μg/kg.min) on the insulin secretory rate into the pancreatico-duodenal vein, and on the glycaemia in the anaesthetized dog. Each point represents the mean of the values obtained in 5 dogs, with the standard error of the mean

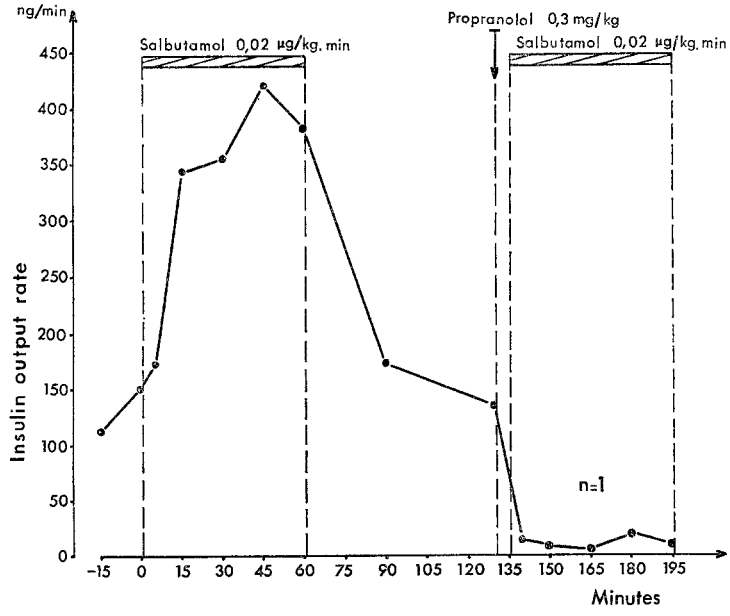


Fig. 6. Modifications of the insulin secretion rate, measured in the pancreatico-duodenal vein in an anaesthetized dog, under the effects, on one hand, of salbutamol alone, and on the other hand, of salbutamol after a preliminary administration of propranolol (0.3 mg/kg I.V.). The results are expressed in ng/min (1 ng = 25 μU)

## Discussion

*I. Demonstration of the stimulating action of the beta-adrenergic receptors on insulin secretion.* The results that we have obtained *in vivo* with the anaesthetized dog, insulin being measured in the pancreaticoduodenal venous blood, enable us to confirm the stimulating effect of isoprenaline on insulin secretion. This effect has already been described in man by Porte [12]. It is worth emphasizing the very important activity of isoprenaline on the secretion of insulin in the dog. Effectively, five minutes after the start of the perfusion, when the total dose administered is 0.075 μg/kg, the stimulating effect was obvious and the secretion was increased 75%.

Previously, we were also able to show the stimulating effect of this drug *in vitro* on the isolated, perfused rat pancreas [10, 8]; although in this case, the stimulating effect of this substance was only temporary and did not persist throughout the entire duration of the administration.

We should note that the doses of DL-isoprenaline used in perfusion were chosen to be sufficiently weak so as not to provoke an elevation of the glycaemia (Fig. 1). In effect, any hyperglycaemia would, by itself, have a stimulating action on the beta cells of the islets of Langerhans, and would interfere with the effects due specifically to isoprenaline. A slight fall in glycaemia actually occurred at the end of the perfusion of isoprenaline; this fall may be related to the hypersecretion of insulin. However, it is probable that this diminution of the glycaemia would have been greater if isoprenaline did not also have a glycogenolytic and hyperglycaemic action [3, 9] as well as its insulin secretory action.

As far as the effects of propranolol alone are concerned (Fig. 2), it is probable that the clear diminution of insulin secretion registered in the first few minutes is due to a suppression of the orthosympathetic tonus that would normally activate the beta-adrenergic receptors of the insulin secretory cells.

The slight fall in glycaemia registered under the effect of propranolol can probably be explained by a diminution of the hepatic glycogenolysis, the activation of the beta-adrenergic receptors being implicated in this mechanism [11, 7].

The administration of isoprenaline after administration of propranolol (Fig. 3) permitted us to ascertain that the latter substance not only inhibited, but even reversed the stimulating effect of the sympathomimetic amine upon insulin secretion. This fact had not, until now, been described *in vivo* but we had already described it *in vitro* in the isolated, perfused rat pancreas [10, 8]. We could perhaps, interpret this phenomenon as being due to the action of isoprenaline on the unblocked alpha-adrenergic receptors remaining free when the beta receptors are blocked by propranolol. Other mechanisms may equally well be involved as will be seen later.

In our experiments, the hypoinsulinaemia noted was accompanied paradoxically, by a considerable reduction in the glycaemia, clearly more important than that provoked by propranolol alone.

In 1967, Porte [12] did not observe any modification of the glycaemia in man with the doses of isoprenaline and propranolol that he used. Under our actual experimental conditions, this important hypoglycaemia is difficult to interpret. In fact, the blood glucose level is due to the intervention of multiple factors, and glycaemic regulation normally constitutes a complex phenomenon. In the present case, it is even more complex due to the actions of the various drugs on the adrenergic receptors, not only of the beta cells in the pancreatic islets, but also of the extrapancreatic tissues (especially those of the hepatic and peripheral tissues). It is important to emphasize this hypoglycaemia provoked by isoprenaline in the presence of propranolol, and to note that the hypoglycaemia can intervene to accentuate the hypoinsulinaemia. In fact, the pancreas is less stimulated because of the reduction of the blood glucose concentration; all hypoglycaemia is accompanied by a decrease in insulin secretion. Moreover, hypoglycaemia, when it is considerable, triggers a secretion of the endogenous catecholamines which are able, by themselves, to accentuate the hypoinsulinaemia by stimulating at the level of the insulin-secretory cell, the alpha adrenergic receptors which remain free.

*II. The characterization of the type of beta-adrenergic receptor responsible for insulin secretion.* If we consider the effects on the insulinaemia of isoprenaline in the presence of practolol, we note an important fact: whereas propranolol suppressed and even reversed the insulin-secretory effect of isoprenaline, practolol did not modify the stimulating action of this sympathomimetic amine (Fig. 4).

Thus, propranolol (global blocking agent of the $\beta_1$ and $\beta_2$ adrenergic receptors) opposed the stimulating insulin-secretory effects of isoprenaline, whereas practolol (quasi-selective blocking agent of the $\beta_1$ adrenergic receptors) (Dunlop and Shanks [5], Barrett *et al.* [2]) displayed no action on the stimulating effects of isoprenaline (activating agent of the $\beta_1$ and $\beta_2$ receptors). Therefore, we are able to acknowledge that the beta-adrenergic receptor involved in insulin secretion may be pharmacologically defined as a $\beta_2$ type.

Nevertheless, this demonstration is based only on an indirect pharmacological argument. Later on we were able to add the direct proof that the insulin secretion consecutive to the stimulation of the beta-adrenergic receptors is of the $\beta_2$ type by using salbutamol, a quasi-selective stimulator of the $\beta_2$ receptors [4,5bis]. As with the administration of isoprenaline, we chose doses which did not provoke any modification of the glycaemia capable of interfering with the effects of the drug itself on the beta insulin secretory cells of the islets of Langerhans.

The recorded results on insulin secretion (Fig. 5) permitted us to ascertain that this drug had a stimulating action as important as that of isoprenaline, the doses used being equally very weak (0.02 μg/kg. min).

Therefore, these experiments reinforce our previous conclusions and confirm that the beta-adrenergic receptors which bring about the stimulation of insulin secretion are of the type $\beta_2$.

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