Biochemical Pharmacology, Vol. 25, pp. 2703–2708. Pergamon Press, 1976. Printed in Great Britain.

PHARMACOLOGICAL COMPOUNDS AFFECTING PLASMA GLUCAGON LEVELS IN RATS*

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(*Received* 25 *February* 1976; *accepted* 21 *June* 1976)

Abstract—Various pharmacological compounds, reserpine, propranolol, hydroquinidine, procaine, verapamil, atropine and nicotinic acid were tested for their ability to modify the glucagon plasma concentration in normal rats both in the basal state and after stimulation by exercise or insulin administration. These drugs were selected on the basis of their known effect on the autonomous nervous system, on transmembrane sodium or calcium fluxes or on the level of plasma free fatty acids. Basal plasma glucagon level was significantly increased 24 hr after administration of reserpine and conversely it was decreased 60 min after administration of hydroquinidine, procaine and atropine. The exercise-induced rise in plasma glucagon which was previously shown to be inhibited by propranolol was not affected by the tested substances with the exception of nicotinic acid. Indeed, blockade of the rise in plasma free fatty acids normally associated with exercise, result in a significantly greater increase in glucagon plasma concentration in animals treated by nicotinic acid. Insulin-induced hypoglycemia provoked a 6-fold increase in plasma glucagon concentration, this rise was not altered by reserpine, hydroquinidine, procaine nor verapamil. It was increased by propranolol which slightly potentiated the hypoglycemic effect of insulin as well as by nicotinic acid which accentuated the depression in plasma free fatty acid induced by insulin. The most striking change was that atropine reduced by about 50 per cent the rise in plasma glucagon secondary to insulin administration. In view of these results, it would be of interest to study the behaviour of plasma glucagon concentration in patients chronically treated with atropine, hydroquinidine or procaine analogs in their respective and already established therapeutic uses.

The finding that glucagon acts as both a hyperglycemic and ketogenic agent in human diabetes mellitus (for review in [1–4]) implies that the research for drugs capable of reducing pancreatic glucagon secretion is necessary. In addition, this type of research may unravel the physiological and biochemical mechanisms controlling glucagon release. In the present study, various compounds were tested for their ability to modify the glucagon plasma concentration in normal rats both in the basal state and after stimulation by exercise or insulin administration.

As previous reports [5, 6] have emphasized the role of the autonomous nervous system in the control of α_2 cell secretion, we investigated the influence of reserpine, propranolol and atropine on glucagon levels. The fact that calcium is involved in the process of insulin [7] and glucagon [8, 9] release prompted us to test the influence of verapamil which has calcium antagonistic properties [10] and of procaine hydrochloride which inhibits the binding and facilitates the release of calcium by phospholipid membranes [11]. Quinidine, which is supposed to reduce outward transport of Na^+ across the cell membrane [12] was tested in light of the crucial role, already documented, that intracellular sodium has in different β cell functions [13–15]. Finally, we tested the effects of an antilipolytic agent, nicotinic acid, in view of the possibility that changes in plasma free fatty acids might modulate the glucagon response to changes in blood glucose [16, 17]. Although the mechanism(s) of action of a given drug can be studied more precisely in *in vitro* systems like isolated islets or isolated perfused pancreas, we tested these compounds *in vivo*, since the final goal of our investigation is the *in vivo* suppression by pharmacological agents of glucagon secretion in human diabetics.

MATERIALS AND METHODS

Animals. Four hundred and ninety-five overnight fasted male albino rats weighing 175–225 g were used in these experiments, according to three different protocols.

(a) The first part of the study was devoted to the effects of the various drugs on glucagon plasma levels under basal conditions; for this purpose we compared animals sacrificed 24 hr or 40 and 60 min after intraperitoneal (i.p.) injection of each drug diluted in saline with controls receiving an equal volume of the solvent.

(b) Groups of rats were compelled to swim for 60 min in tepid water according to a previously described procedure [6, 18] after either intraperitoneal administration of saline or injection of the drug to be tested.

(c) Acute hypoglycemia was induced by i.p. injection of 2.5 U regular insulin (Actrapid M.C. Novo, Copenhagen, Denmark) per kg body wt. Here again control animals received insulin with saline whereas seven groups of 7–16 rats were given insulin and one

* Part of these data were presented at the Tenth Annual Meeting of the European Association for the Study of Diabetes. Jerusalem, Sept. 1974 and published in Abstract form: *Diabetologia* **10**, 378 (1974).

† Chercheur Qualifié of the Fonds National Belge de la Recherche Scientifique.

Table 1. Influence of insulin administration and physical exercise (forced swim) on blood glucose, plasma free fatty acids and glucagon in saline-injected control rats. (mean ± S.E.M., n = number of animals)

	Blood glucose (mg %)	Plasma FFA (μeq/l)	Plasma glucagon (pg/ml)
Controls (40 min after saline) (n = 54)	77.8 ± 1.4	987 ± 46	114.1 ± 4.6
Insulin-induced hypoglycemia (40 min after 2.5 U/kg Actrapid insulin) (n = 54)	30.0 ± 0.9 P < 0.001	437 ± 20 P < 0.001	753.4 ± 63.6 P < 0.001
Insulin-induced hypoglycemia (60 min after 2.5 U/kg Actrapid insulin) (n = 6)	33.2 ± 2.5 P < 0.001	557 ± 79 P < 0.005	679 ± 91.6 P < 0.001
Controls (60 min after saline) (n = 97)	75.7 ± 1.3	1004 ± 43	124.9 ± 5.3
Muscular exercise (60 min forced swim after saline) (n = 44)	81.4 ± 2.2	1960 ± 120 P < 0.001	224.4 ± 12.9 P < 0.001

of the compounds studied. Animals were sacrificed 40 min after insulin administration. In all cases, the rats were stunned by a blow on the head and blood was withdrawn into heparinized glass syringes by cardiac puncture and immediately transferred to chilled graduated tubes. Trasylol®* (5000 U/ml) supplemented with EDTA (12 mg/ml) was immediately added to each sample (0.1 ml of the mixture per ml of blood).

Methods. Blood glucose was determined according to the enzymatic method using glucose-oxidase (Biochemica Test 15756 B Boehringer, Mannheim, W. Germany). The concentration of plasma FFA was measured by the titrimetric method of Dole and Meinertz [19]. Plasma insulin was determined in duplicate by the C method of Hales and Randle [20] using centrifugation instead of filtration and rat insulin (kindly given by Dr. Schlichtkrull, Novo Industri AS Copenhagen) as standard. Glucagon was measured in duplicates by a radioimmunoassay technique [21] using dextran-charcoal for the separation of free and bound hormone. Antiserum 30K, considered to be specific for glucagon was used in all the determinations and crystalline pork glucagon (lot 6770 MC) from Novo Industri AS (Copenhagen) was used as standard.

Pharmacological compounds. Reserpine (Serpasil®, Ciba, Basel, Switzerland) was administered i.p. at a dose of 10 mg/kg body wt 24 hr before sacrifice.

The following agents were given i.p. at their respective dosages 40 or 60 min before sampling: propranolol hydrochloride (Inderal®, I.C.I., Macclesfield, England) 10 mg/kg; dihydroquinidine gluconate (Hydroquinidine®, Houdé, Paris, France) 5 mg/kg; procaine hydrochloride (Bios, Brussels, Belgium) 50 mg/kg; verapamil hydrochloride (Isoptine®, Knoll A.G., Ludwigshafen, Germany) 0.5 mg/kg; atropine sulfate (Biergon, Herstal, Belgium) 0.2 mg/kg and nicotinic acid (Niacin®, U.C.B., Brussels, Belgium) 5 mg/kg.

Statistical methods. Results were expressed as mean ± S.E.M. and the statistical significance of the observed differences was analysed by use of Student's

*Trasylol, aprotinin BAYER (Leverkusen, Germany) prevents glucagon degradation by plasma proteases [21].

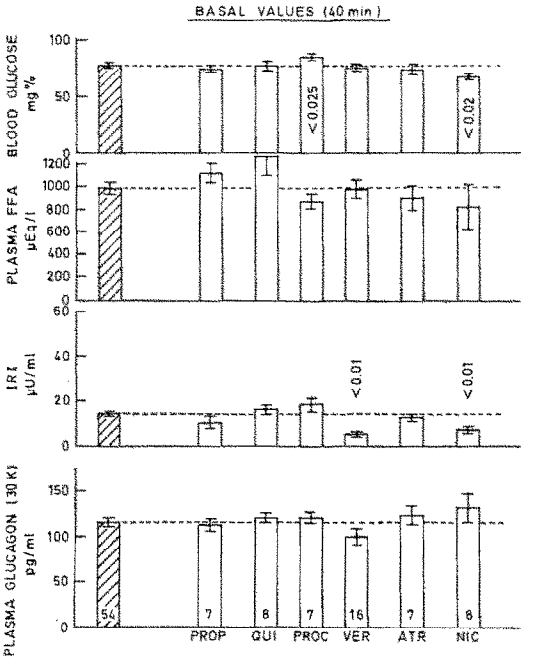

Fig. 1. Influence of propranolol (PROP), hydroquinidine (QUI), procaine (PROC), verapamil (VER), atropine (ATR) and nicotinic acid (NIC) on blood glucose, plasma FFA, insulin and glucagon in overnight fasted rats. Control animals (hatched column) received saline and all rats were sacrificed 40 min after intraperitoneal injection of saline or of the drugs. The height of each column corresponds to the mean ± S.E.M., the number of animals in each series being indicated inside the lower column. A statistically significant difference for comparison with control animals is indicated by the value of P, inside or at the top of the corresponding column.

t-test for non-paired values. The coefficient of probability P and the correlation coefficient were obtained according to Snedecor [22].

RESULTS

1. *Control animals.* Table 1 summarizes the mean results of blood glucose, plasma FFA and glucagon determinations in the groups of saline-injected control rats receiving insulin or submitted to muscular exercise. Insulin administration resulted in a marked decrease in blood glucose and plasma FFA associated with a 6-fold increase in plasma glucagon 40 and 60 min after the insulin injection. At 40 min, there was no correlation between the values of plasma glucagon and those of the corresponding blood glucose and plasma FFA ($r = 0.21$ and $r = 0.10$ respectively, $n = 54$, $P > 0.1$).

Exercise significantly increased FFA and glucagon plasma concentrations whereas blood glucose remained unchanged.

2. *Animals receiving the various drugs in the basal state.* Figures 1 and 2 compare the mean values of blood glucose and FFA, insulin and glucagon plasma concentrations measured 24 hr after reserpine and 40 and 60 min after administration of propranolol, hydroquinidine, procaine, verapamil, atropine and nicotinic acid with the mean corresponding values obtained in saline-injected control rats.

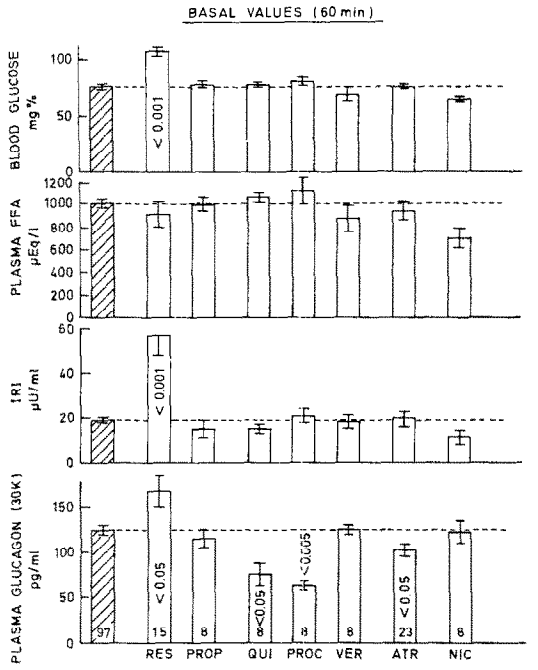

Fig. 2. Influence of reserpine (RES), propranolol (PROP), hydroquinidine (QUI), procaine (PROC), verapamil (VER), atropine (ATR) and nicotinic acid (NIC) on blood glucose, plasma FFA, insulin and glucagon in overnight fasted rats. With the exception of the RES group which received reserpine (10 mg/kg body wt) 24 hr before sacrifice and saline 60 min before sacrifice, control animals (hatched column) as well as the experimental groups were sacrificed 60 min after intraperitoneal injection of saline or of the tested drug. See legend to Fig. 1 for additional information.

Pretreatment with reserpine significantly increased blood glucose (+31 mg/100 ml), plasma insulin (+38 µU/ml) and plasma glucagon (+54 pg/ml) in relation to the saline injected controls while plasma FFA remained unchanged. In the propranolol treated groups, none of the values of blood glucose, plasma FFA, insulin and glucagon levels significantly differed from basal concentrations 40 and 60 min after injection.

Hydroquinidine and procaine significantly decreased plasma glucagon level at 60 min (−50 pg/ml and −62.5 pg/ml, respectively) without affecting blood glucose, plasma FFA and insulin at this time; the only change seen at 40 min was a modest although statistically significant increase in blood glucose (+8 mg/100 ml) with procaine.

Verapamil transiently depressed plasma IRI at 40 min (−9 µU/ml), but did not modify the other parameters. With atropine, a single modification was noted, namely a modest decrease in plasma glucagon at 60 min (−22.5 pg/ml). Finally, nicotinic acid administration was followed by a decrease in blood glucose (−10 mg/ml) and plasma insulin (−7 µU/ml) at 40 min. These changes persisted at 60 min together with a trend towards a decrease in plasma FFA (−285 µeq/l) which was weakly significant ($P < 0.05$). Plasma glucagon was not affected.

3. Insulin-induced hypoglycemia. The results of blood glucose, plasma FFA and glucagon determinations 40 min after i.p. injection of 2.5 U regular insulin per kg body wt are depicted in Fig. 3. When compared with the mean value of blood glucose measured in the control group 40 min after administration of insulin alone (30.0 ± 0.9 mg/100 ml, n = 54), the blood sugar levels found in animals pretreated with reserpine (51.4 ± 4.4 mg/100 ml, n = 8) hydroquinidine (41.2 ± 2.7 mg/100 ml, n = 8) and procaine (38.0 ± 2.8 mg/100 ml, n = 8) were statistically higher, whereas the result found in rats pretreated with propranolol (24.8 ± 1.7 mg/100 ml) was statistically lower. The FFA-lowering action of insulin was significantly potentiated by reserpine, verapamil and nicotinic acid and, on the contrary, was reduced by propranolol. Forty min after administration of insulin alone to the control group, plasma glucagon was increased to 753.4 ± 63.6 pg/ml (see Table 1). This value was similar to those found in animals receiving insulin and reserpine or hydroquinidine or procaine or verapamil. The glucagon increase was exaggerated when insulin was associated with propranolol (1388 ± 190 pg/ml) or with nicotinic acid (1406 ± 200 pg/ml). On the contrary, atropine markedly reduced the stimulatory effect of insulin-induced hypoglycemia both at 40 min (Fig. 3) and at 60 min (Fig. 4). Figure 4 also shows that this reduction of glucagon by atropine was associated with a more profound depression of plasma FFA.

4. *Muscular exercise.* The results of blood glucose, plasma FFA and glucagon determinations after 60 min forced swim are depicted in Fig. 5. In 44 saline-injected exercised animals, blood glucose averaged 81.4 mg/100 ml (± 2.2 S.E.M.), plasma FFA averaged 1960 µeq/l (± 120 S.E.M.) and plasma glucagon

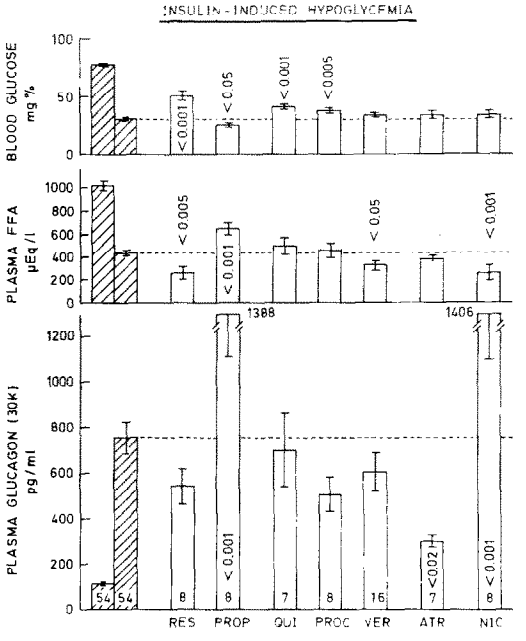

Fig. 3. Influence of reserpine (RES), propranolol (PROP), hydroquinidine (QUI), procaine (PROC), verapamil (VER), atropine (ATR) and nicotinic acid (NIC) on blood glucose, plasma FFA and glucagon 40 min after insulin administration. The first hatched column corresponds to saline-injected animals and the second hatched column to the animals receiving insulin alone used for statistical comparison. See legend to Fig. 1 for additional information.

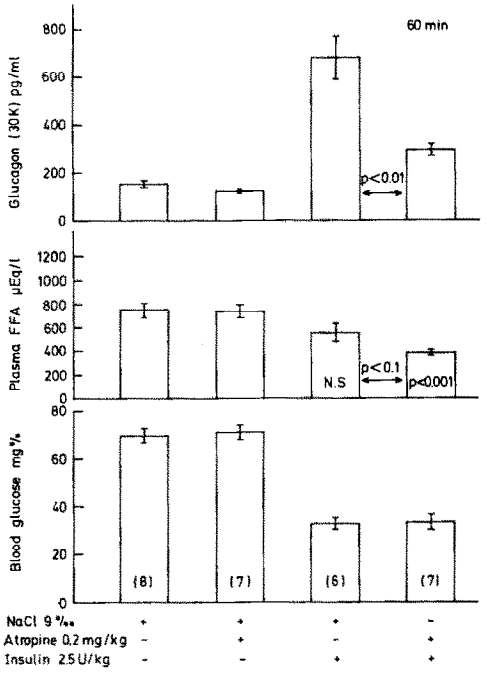

Fig. 4. Effect of the intraperitoneal injection of atropine, insulin and atropine plus insulin on blood glucose, plasma FFA and glucagon in overnight fasted rats. The control group received saline. All animals were sacrificed 60 min after the injection. See legend to Fig. 1 for additional information.

averaged 224.4 pg/ml (±12.9 S.E.M.). Thus, the increase in plasma glucagon in the present series averaged 99.5 pg/ml. In five separate experiments comparing resting and exercised rats [6], the mean increase in plasma glucagon was 113.9 pg/ml (extreme values 93.6–134.0 pg/ml, S.D. 19.2; S.E.M. 8.6). In rats pretreated with reserpine, the post-exercise values of blood glucose and plasma glucagon were higher than in control exercised rats. However, comparison with the pre-exercise values revealed that these apparent changes were entirely due to the effects of reserpine on basal values (see Fig. 2). Indeed, in animals given reserpine, exercise *per se* did not significantly modify blood glucose and the increase in plasma glucagon associated with exercise was +147.5 pg/ml, a value similar to those found in untreated animals. Although statistically significant, the rise in plasma FFA associated with exercise was diminished in reserpine-treated rats.

Neither hydroquinidine nor procaine, verapamil and atropine significantly affected the post-exercise values of blood glucose, plasma FFA and glucagon. Administration of nicotinic acid immediately before swimming abolished the rise in plasma FFA and markedly potentiated the glucagon rise (+243.1 pg/ml) associated with exercise.

DISCUSSION

Numerous arguments have accumulated during recent years in favor of attributing to glucagon a role as a hyperglycemic and ketogenic factor in human diabetes mellitus [1–4]. This justifies effort to try to find pharmacological compounds capable of reducing the glucagon hypersecretion associated with most cases of decreased glucose tolerance or increased ketogenesis. Although somatostatin and its analogs are the most promising agents in this field [23, 24], the short half-life of this peptide as well as its possible untowards effects on platelet functions [25–27] must be taken into consideration. Glucagon secretion is stimulated by catecholamines *in vitro* [28] and *in vivo* [29] and the results of most studies are compatible with the view that the adrenergic receptors involved in alpha cell stimulation are of the β-type. Indeed, propranolol pretreatment abolished the exercise-induced glucagon rise in rats [18]. The present work demonstrates that propranolol, even at high doses, does not alter basal glucagon concentrations 40 or 60 min after its injection. In agreement with Baird and Carter [30] we noticed that propranolol, when combined with insulin gave rise to blood glucose levels which were lower than those produced by insulin alone. Although very small (5.2 mg/100 ml), the mean additional reduction in blood glucose provoked by propranolol is perhaps responsible for the higher glucagon levels measured under these conditions. This finding shows that sympathetic efferent activity is apparently not responsible for the glucagon response to hypoglycemia in rats, a conclusion already drawn by Bloom *et al.* [5] in the calf.

Reserpine administration depletes the catecholamine stores of the adrenals and the noradrenaline stores of adrenergic nerve endings [31]. Twenty-four hr after administration of this agent, treated animals exhibited moderate hyperglycemia and hyperglucagonemia and marked hyperinsulinemia. This basal hyperglucagonemia, the mechanism of which is unknown, can entirely account for the higher glucagon

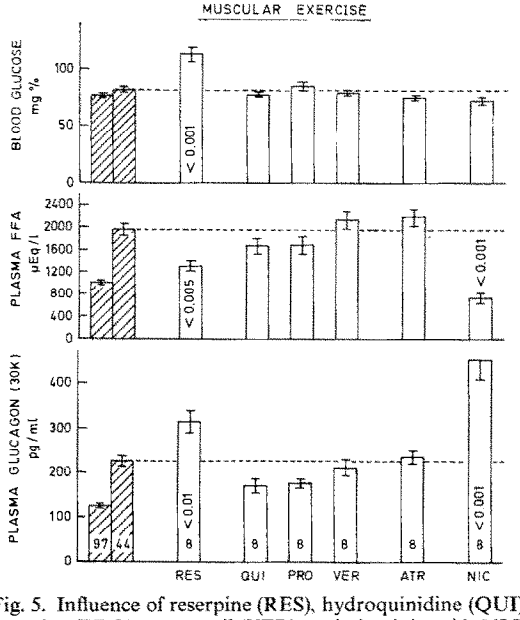

Fig. 5. Influence of reserpine (RES), hydroquinidine (QUI), procaine (PRO), verapamil (VER), and nicotinic acid (NIC) on blood glucose, plasma FFA and glucagon after 60 min forced swim. The first hatched column corresponds to the saline-injected resting animals and the second hatched column to the saline-injected exercised rats used for statistical comparison. See legend to Fig. 1 for additional information.

plasma levels measured after exercise. Indeed, the glucagon rise associated with exercise in reserpine-treated rats was comparable to that seen in control animals. Finally, the normal glucagon increase associated with insulin-induced hypoglycemia rules out any role for reserpine as an inhibitor of glucagon secretion. In agreement with Taylor [32], we found that reserpinized rats exhibited a significant increase in resting blood glucose, which may be secondary to the hyperglucagonemia demonstrated in this study. Although reduced, exercise-induced FFA mobilization persisted in exercised animals treated with reserpine, suggesting either that catecholamine depletion was not complete or that exercise-induced lipolysis was partially independent from catecholamines.

The present study demonstrates that hydroquinidine transiently depresses basal glucagon levels but does not alter the rise in plasma glucagon associated with muscular exercise or insulin hypoglycemia. The reason why the hypoglycemia induced by insulin is less marked in the presence of hydroquinidine is unknown and merits further investigation. Procaine hydrochloride is a local anesthetic which alters cell membrane handling of calcium. According to Hope-Gill *et al.* [11] both insulin and procaine inhibit binding and facilitate release of calcium by phospholipid membranes and the decrease in membrane calcium might increase permeability of these membranes to glucose. These mechanisms could explain the 22 per cent decrease in basal plasma glucagon seen in rats treated with procaine. This weak inhibitory effect of procaine on glucagon secretion is however insufficient to affect the stimulation of α cells by exercise or hypoglycemia. Verapamil has been shown to inhibit glucose-induced insulin release by the isolated perfused rat pancreas probably by interfering with calcium transport across the plasma membrane [10]. The significant decrease in basal plasma insulin in rats treated with this drug is thus in agreement with the previously published results obtained *in vitro*. Under our experimental conditions, verapamil did not affect glucagon plasma levels under any of the tested conditions. Blood glucose was not altered and the single significant change was a potentiation of the FFA-lowering effect of insulin.

Our results demonstrate the importance of the parasympathetic innervation of the islets of Langerhans in the control of glucagon secretion in rats. Indeed, administration of atropine not only decreased basal plasma glucagon concentration but also markedly reduced the stimulatory action of insulin-induced hypoglycemia on glucagon secretion. These data are in agreement with previous observations in calves [5] and in man [33].

It can thus be concluded that the *in vivo* stimulation of glucagon release is mediated not only by glucopenia at the alpha cell level but also by a cholinergic mechanism secondary to vagal stimulation. It is worthwhile to emphasize the importance of the parasympathetic system since relatively few studies have been conducted in this field compared with those devoted to the adrenergic system.

Nicotinic acid is an antilipolytic agent used in the therapy of various types of hyperlipoproteinaemia [34]. Previous reports have emphasized the important role free fatty acids perform in dogs [35] and in man [17] in the control of glucagon secretion. The hypothesis can thus be put forward that chronic treatment with nicotinic acid derivatives would reduce plasma FFA leading to chronic stimulation of glucagon secretion and secondarily to impairment of glucose tolerance. In fact, hyperglucagonemia is seen in various cases of hyperlipoproteinemia [36] and a gross and sustained rise in fasting plasma glucagon was previously reported in hyperlipidemic patients treated with β-piridyl-carbinol, a nicotinic acid derivative [37, 38].

In the present study, we noticed that with pre-treatment with nicotinic acid, elevation in plasma FFA normally seen during exercise did not occur, and that exercise-induced hyperglucagonemia was potentiated. Just as insulin and nicotinic acid produced a fall in plasma FFA which was deeper than that seen following insulin alone, the hyperglucagonemia induced by insulin and nicotinic acid was greater than that produced by insulin alone. These results provide evidence that the stimulation of glucagon secretion by exercise or hypoglycemia can be modulated by the level of plasma FFA. Careful studies of plasma glucagon in patients treated with nicotinic acid should also be carried out to assess the possible role of glucagon in the impairment of glucose tolerance associated with nicotinic acid administration.

Acknowledgements—We are indebted to Dr. R. H. Unger (Dallas) for the supply of 30K antiserum, to Mrs. Cartenstadt, Mr. and Mrs. Lallemand and Miss Rombaux for their skilled technical assistance, to D. Binder for linguistic assistance and to Mrs. Vaessen-Petit for secretarial help.

This work was supported by the Fonds de la Recherche Scientifique Médicale Belge.

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