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EFFECT OF OUABAIN ON INSULIN SECRETION IN THE ANESTHETIZED DOG*

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Abstract—The effect of ouabain on insulin secretion has been studied in the anesthetized dog. Blood glucose, plasma free fatty acids and plasma amino nitrogen were determined simultaneously. When infused at a dose of 1 μg/kg/min, ouabain significantly reduces blood glucose and plasma free fatty acid levels and increases plasma amino nitrogen. Plasma insulin concentrations are significantly increased in both arterial and pancreaticoduodenal venous blood. The pancreaticoduodenal vein blood flow is not significantly modified. Calculated pancreatic insulin production is markedly increased during ouabain infusion. These findings were confirmed using "one shot" injections of ouabain. The mechanism of the marked *in vivo* effect of ouabain on insulin production is discussed.

A STIMULATORY effect of ouabain on insulin secretion *in vitro* was first described in 1967 by Milner and Hales[1] on pieces of rabbit pancreas. Since then, such an effect has either failed to be confirmed[2,3] or has been detected only with huge concentrations of ouabain[4] or crucial concentrations of glucose.

In vivo, however, two groups working on the dog have been able to demonstrate an increase in portal plasma insulin concentration[6,7] and in pancreaticoduodenal insulin production[8] during the intravenous infusion of much lower doses of ouabain.

While *in vitro* experiments represents the most widely used technique for studying the mechanism of insulin secretion, we believe that they are most valuable when confirmed under *in vivo* conditions. *In vitro* experiments have led to the conclusion that ionic flux across the β cell membrane—probably the sodium and calcium fluxes primarily—are important events in initiating or modulating the insulin secretory process.[1,5] In this connection, the effect of ouabain, a classical inhibitior of the Na/K ATPase-dependent sodium pump, on insulin secretion, deserves special consideration, particularly if it can be observed *in vivo*. We thus focused our investigation on the effects of ouabain on insulin secretion and circulating levels of certain crucial metabolites (glucose, free fatty acids, plasma amino nitrogen) in the anesthetized dog.

MATERIALS AND METHODS

Mongrel dogs weighing about 15 kg and fasted overnight were used for the experiments. They were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg body wt.). Body temperature was kept constant with warming blankets. The trachea was canulated and the animals were given a mixture of O_2 95%–CO_2 5%

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to breathe freely. At laparotomy, an appropriate size polyethylene catheter was introduced into the proximal part of the superior pancreaticoduodenal vein (PDV) at about 2 cm from the portal vein. The PDV was then ligated, and the pancreatic venous effluent was drained off and reinfused through the splenic vein as previously described.[9] This permitted measurement of pancreatic blood flow. Throughout the experiments, the animals were infused intravenously with saline (1 ml/min).

Femoral blood pressure (mercury manometer), pulse rate and PDV blood flow were recorded every 5–10 min. Blood samples were drawn simultaneously from the femoral artery (9 ml) and, by free flow, from the PDV (4 ml). Collected blood was immediately replaced by an equivalent amount of blood obtained from a donor. Thirty to 45 min after the end of the surgical procedure, three blood samples were drawn at 7·5 min intervals to provide basal values. From zero time, an additional intravenous saline infusion (1 ml/min) was started in seven animals, ouabain infusion (1 μg/kg body wt./min in 1 ml/min of saline) was started for 1 hr in six dogs, and "shot injection" of ouabain (10 μg/kg body wt.) injected in 1 min in a volume of 10 ml of saline) was effected in two additional animals. Blood samples were drawn from the femoral artery and PDV every 10 min during the perfusion of saline or ouabain an then 15 and 30 min after the termination of perfusion. In shot injection experiments, blood samples were drawn 1, 3, 5, 10, 15, 20 and 25 min after the end of the injection.

Glucose concentrations in arterial blood were measured by the method of Hoffman[10] adapted to the AutoAnalyzer (Technicon), and free fatty acids (FFA) on arterial plasma were measured by the method of Dole and Meinertz.[11] Plasma amino nitrogen was determined according to Malangeau *et al.*[12] Plasma insulin was assayed by a modification[13] of the double antibody method of Morgan and Lazarow,[14] using an anti-pig insulin antiserum. Human insulin was used as a standard and all samples from a single experiment were assayed in the same series using the same standard curve. The identity of reactivity between dog and human insulins was assayed by verifying the parallelism of dilution curves. At the end of the experiment, the animal was killed and the part of the pancreas studied in the experiment was determined by retrograde infusion of 10–15 ml of a 1% aqueous solution of methylene blue in the PDV catheter, according to the procedure described by Seltzer.[15]

RESULTS

(1) When infused at a dose of 1 μg/kg/min, ouabain induced only a moderate decrease in heart rate and some slight statistically insignificant changes in systolic blood pressure and pancreaticoduodenal vein blood flow (Fig. 1).

(2) Under the same experimental conditions, ouabain significantly reduced blood glucose and plasma free fatty acid levels; a small but significant rise in plasma amino nitrogen was observed at the end of the infusion and during the next 30 min (Fig. 2).

(3) As indicated in Fig. 3, immunoreactive insulin levels rose markedly in both arterial and pancreaticoduodenal vein plasmas in response to ouabain infusion. Taking into account the blood flow, the ouabain-induced rise in insulin output was highly significant (Fig. 4) and differed statistically from the small spontaneous rise observed in the late phase of control experiments in which only saline was perfused (Fig. 5). The clear-cut rise in insulin production was confirmed when ouabain was administered in a "one-shot" experiment (Fig. 6).

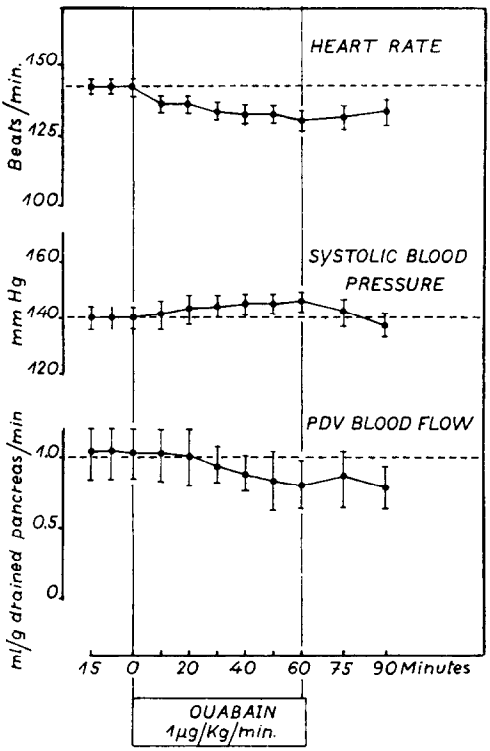

FIG. 1. Hemodynamic changes induced by the intravenous infusion of ouabain in six anesthetized dogs. Results are expressed as mean ± S.E.M.

DISCUSSION

The experiments reported herein extend and confirm the results of Loubatieres *et al.*[8] and Triner *et al.*[6,7] A clear-cut stimulatory effect of ouabain on the production of insulin in the anesthetized dog has been demonstrated in both infusion and "one shot" experiments. It seems reasonable to consider the decline in blood glucose and plasma FFA a consequence of the ouabain-induced enhancement of insulin production. The later rise in plasma amino nitrogen is more difficult to interpret and requires further investigation.

The unequivocal stimulation of insulin production by ouabain in the dog runs counter to the discrepancies of the *in vitro* experiments, in which much higher concentrations of ouabain were necessary to enhance insulin release even inconstantly (see Introduction). As suggested by Triner *et al.*,[7] the explanation may lie in the high affinity of the pancreas for cardiotonic heterosides: Doherty *et al.*[16] have demonstrated that the pancreatic affinity for digoxin is second only to that of the heart, and that, under conditions of serum plateau, the mean pancreas to serum digoxin ratio is about 10. Under these conditions, the concentration of the cardiotonic heteroside in the pancreas (and perhaps even more in certain pancreatic cells such as the insulin-producing cells of the islets) might be particularly high during *in vivo* infusion.

The mechanism of the stimulation of insulin secretion by ouabain remains unknown. Triner *et al.*[7] have suggested that the increase in portal plasma insulin concentrations

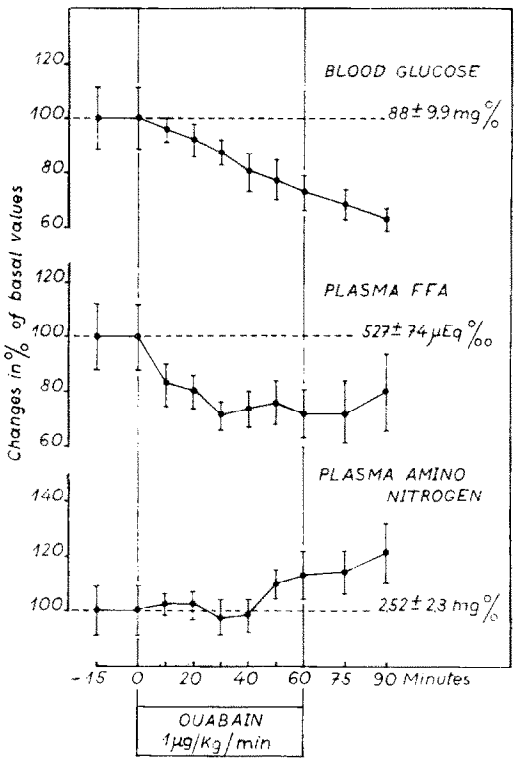

FIG. 2. Metabolic changes induced by the intravenous infusion of ouabain in six anesthetized dogs. Results are expressed as mean ± S.E.M. in percent of basal values. Mean absolute basal values are indicated for each parameter.

might be due to a redistribution of the cardiac output so as to favor the pancreatic circulation. However, this possibility is ruled out by our observation that ouabain does not significantly affect pancreatic blood flow, indicating that the rise in insulin levels reflects a true increase in insulin production; nevertheless, the possibility still remains that ouabain might affect the microcirculation within the islets[17] and thereby modify insulin secretion. The suggestion that the increase in insulin levels might be due to the sugar molecule which is part of the cardiac glycoside seems extremely unlikely in view of the very low amount of "sugar" infused during these experiments (0·3 mg of rhamnose in 1 hr in a 20-kg dog). It seems most likely that the increase results from an action of ouabain on the β cell either *via* changes in ionic movements due to the inhibition of the sodium pump or *via* a direct effect of ouabain on enzyme systems which control insulin secretion.

In connection with the first mechanism, it has been suggested that depolarization of the β cell and Na^+ influx might lead to insulin secretion.[1,5,18] In this respect, inhibition of the β cell sodium pump by ouabain may facilitate Na^+ influx and hence insulin secretion. As recently discussed in detail by Malaisse *et al.*[5], however, strong experimental evidence is now bolstering the hypothesis that calcium rather than sodium uptake is a crucial event in the sequence of phenomena leading to insulin release. Independently of any hypothetical direct effect of ouabain on calcium uptake,

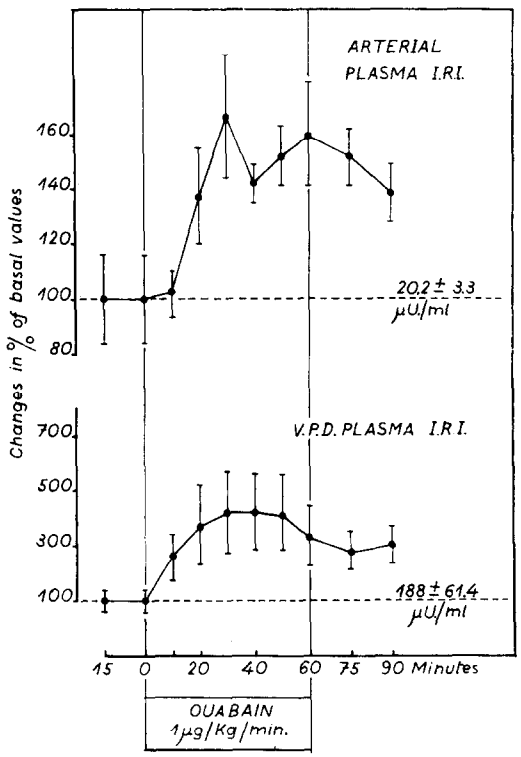

FIG. 3. Changes in arterial and pancreaticoduodenal vein plasma insulin concentrations induced in six anesthetized dogs by the intravenous infusion of ouabain. Results are expressed as mean ± S.E.M. in per cent of basal values.

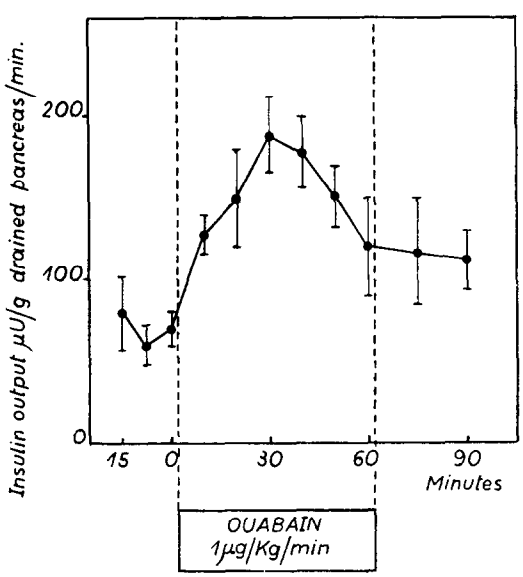

FIG. 4. Changes in pancreatic insulin output induced in six anesthetized dogs by the intravenous infusion of ouabain (mean ± S.E.M.).

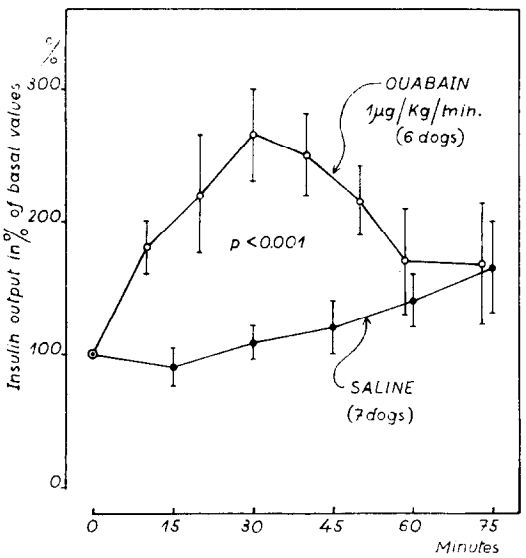

FIG. 5. Changes in pancreatic insulin output in six ouabain-infused dogs compared to the changes observed in a series of seven control dogs infused with saline only. In the control series, saline was infused intravenously during all the experiment (2 ml/min from 0 to 60 min; 1 ml/min later). In the ouabain series, saline (1 ml/min) was infused from 0 to 75 min, ouabain was infused from 0 to 60 min and the appropriate dose was diluted in 1 ml of saline. Results are expressed as mean ± S.E.M. in per cent of basal values.

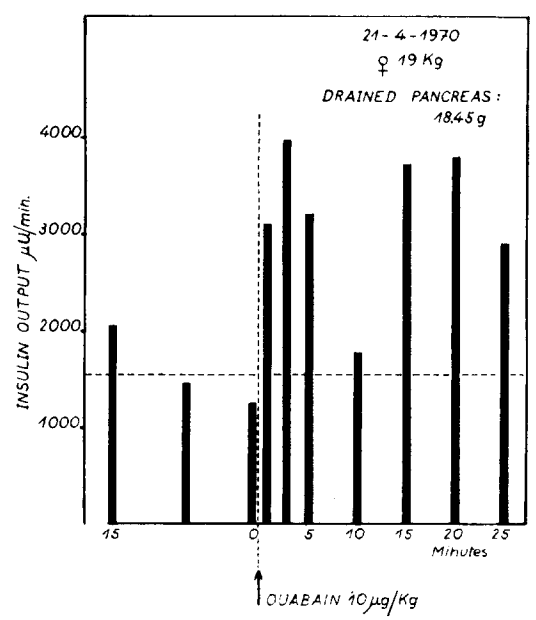

FIG. 6. Changes in pancreatic insulin output induced by a "one-shot" intravenous injection of ouabain (10 µg/kg) in an anesthetized dog.

we should recall that several observations indicate that changes in sodium flux across the cell membrane affect the transmembrane flux and intracellular distribution of calcium.[19,20] We may therefore speculate that the effect of ouabain on insulin secretion might be indirectly due to changes in calcium uptake by (or calcium movements in) the β cell.

The potent effect of ouabain on insulin secretion, observed in dogs at doses only two to four times higher than those used for therapeutic purposes in man, raises the question of whether insulin release and subsequent hypoglycemia may occur under certain conditions of massive cardiotonic heteroside therapy or during poisoning by these compounds. A clinical investigation of this possibility is indicated.

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REFERENCES

1. R. D. G. Milner and C. N. Hales, *Biochim. biophys. Acta* **135,** 375 (1967).
2. S. L. Howell and K. W. Taylor, *Biochem. J.* **108,** 17 (1968).
3. W. J. Malaisse, *Etude de la Sécrétion Insulinique* in vitro. Ed. Arscia, Brussels (1969).
4. A. E. Lambert, Biochemical and morphological studies of cultured foetal rat pancreas. Thesis. University of Louvain (1970).
5. W. J. Malaisse, F. Malaisse-Lagae and G. Brisson, *Horm. Metab. Res.* **3,** 65 (1971).
6. L. Triner, P. Killian and G. G. Nahas, *Science* **162,** 560 (1968).
7. L. Triner, J. Papayoanou, P. Killian, Y. Vulliemoz, R. Castany and G. G. Nahas, *Circulat. Res.* **25,** 119 (1969).
8. A. Loubatieres, M. M. Mariani, L. Savi and G. Sorel, *C.R. Soc. Biol.* **162,** 1589 (1968).
9. P. Lefebvre and A. Luyckx, *Archs int. Physiol. Biochem.* **74,** 867 (1966).
10. W. S. Hoffman, *J. biol. Chem.* **120,** 51 (1937).
11. V. P. Dole and H. Meinertz, *J. biol. Chem.* **235,** 2395 (1960).
12. P. Malangeau, R. Bourdon, A. M. Nicaise and B. Masson, *Ann. Biol. Clin.* **21,** 1 (1963).
13. H. J. Quabbe, *Diabetologia* **5,** 101 (1969).
14. C. R. Morgan and A. Lazarow, *Diabetes* **12,** 115 (1963).
15. H. S. Seltzer, *J. clin. Invest.* **41,** 289 (1962).
16. J. E. Doherty and W. H. Perkins, *with the assistance of* J. Gamill, J. Sherwood and C. Dodd, *Am. J. Cardiol.* **17,** 47 (1966).
17. J. E. Vance, *Diabetes* **19,** 402 (1970).
18. C. N. Hales and R. D. G. Milner, *J. Physiol.* **194,** 725 (1968).
19. R. F. Palmer and V. A. Posey, *J. gen. Physiol.* **50,** 2085 (1967).
20. L. L. Simpson, *J. Pharm. Pharmac.* **20,** 889 (1968).