Hypoglycemic Effect of Ouabain in Dogs* (32923)

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It has been reported that ouabain exerts *in vitro* metabolic effects characterized by an inhibition of spontaneous (1) and epinephrine-stimulated glycogenolysis (2,3) and lipolysis (2,4,5). All these *in vitro* studies were performed with high concentrations of ouabain (10^{-5} *M* to 10^{-4} *M*). The purpose of the present study was to determine if ouabain exerted metabolic effects in intact animals in a dosage much lower than that used *in vitro*, and if a similar interaction with the metabolic effect of epinephrine occurred. Assuming that the total dose of ouabain administered to the animals (1 μg/kg per min for 60 min) accumulated in a volume of water approximating extracellular water, the final concentration would be about 10^{-7} *M*. This dose is at least 100 times less than that used *in vitro*.

Methods. Experiments were performed on 14 pedigreed male beagles less than 1 year old and fasted for 18 hours before the experiment. The animals were anesthetized with 35 mg/kg of Na pentobarbital, intravenously administered, and intubated with a cuffed endotracheal tube. Mechanical ventilation with 100% O_2 was administered at constant rate and volume so as to maintain normal acid–base equilibrium and O_2 saturation in arterial blood throughout the experiment. Muscle relaxation was maintained with succinylcholine chloride. Each animal served as his own control and was studied twice at no less than a week's interval according to a protocol previously described (6).

Two series of experiments were performed. In the first series of experiments on 6 dogs, following the control period, ouabain (1.0 μg in 0.1 ml of saline/kg per min) was administered i.v. for 60 min. Observations were continued for 60 min following the end of ouabain administration. In the control experiment, the same volume of saline as of ouabain was administered. In the second series of experiments on 8 dogs, the effect of ouabain on the metabolic activity of epinephrine was studied. After the control period, ouabain (1.0 μg/kg per min) was administered to the animal for 60 min in the same volume as given in the first series. During the last 30 min of this infusion, epinephrine (0.8 μg in 0.1 ml of saline/kg per min) was administered intravenously. Observations were continued for 60 min following the end of drug infusion. In the control experiment performed on the same animal, a volume of isotonic saline equal to that of ouabain was given while the same dose of epinephrine was administered. As in the first series, the sequence of ouabain–saline administration was reversed from one animal to the next. Specific methods of measurements have been described in previous publications (6). The data was analyzed statistically according to the Student *t* test. The significance of differences between paired measurements was calculated by the method of difference between correlated pairs. The *p* was evaluated within 5% limit of confidence.

Results. Ouabain administration. Mean blood pressure increased at the end of ouabain infusion and returned to control levels 60 min after the end of infusion. Heart rate did not change substantially throughout the experimental period and did not differ from rates observed during the control experiment. The ECG patterns were not altered; arterial pH and pCO_2 remained within the normal range throughout the experiment. During the infusion of ouabain, blood glucose decreased gradually from a control value of 101 mg/100 ml and reached its lowest level (72 mg/100 ml) 30 min after the end of the infusion (a

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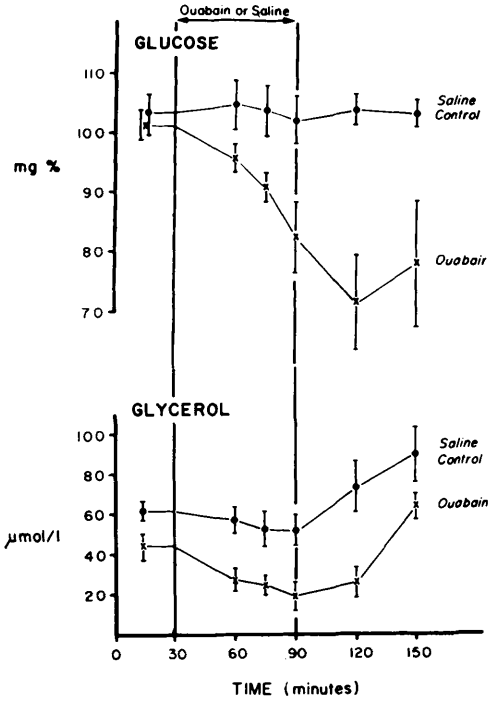

FIG. 1. Changes in blood glucose and glycerol concentrations in control (saline infusion) and ouabain-treated beagles. Means ± SE of 6 experiments are given.

30% decrease, $p<0.05$) (Fig. 1). Sixty min after the end of the infusion, glucose concentration had returned close to its control value. Plasma glycerol dropped from a control level of 43.8 μmole/liter to 18.3 μmole/liter at the end of the infusion (a 55% decrease, $p<0.05$). Plasma free fatty acids (FFA) decreased consistently during ouabain infusion but, because of the greater variability of the response, the change was not significantly different from the control group. The O_2 uptake did not change significantly during ouabain administration, nor did plasma K^+ concentration.

Ouabain and epinephrine administration. During epinephrine infusion, blood glucose increased by 126 mg/100 ml in control dogs and by 95 mg/100 ml in ouabain-treated dogs (Fig. 2). This increase from pre-epinephrine levels in the ouabain-treated dogs is significantly lower (—24%, $p<0.05$) than that seen in the control animals, although the glucose concentrations at the end of epinephrine infusion are not significantly different. Plasma glycerol increased by 155 μmole/liter in control dogs and by 67 μmole/liter in ouabain-treated dogs (—57%, $p<0.01$). Plasma FFA increased by 810 μeq/liter in the control group and by 240 μeq/liter in ouabain-treated dogs (—67%, $p<0.05$). The O_2 uptake increased significantly and to the same extent during and 30 min after epinephrine infusion in both groups. Plasma K^+ concentration did not change significantly.

Discussion. The hypoglycemic effect of ouabain could be mediated through a stimulation of insulin production, a hypothesis which will have to be tested by further experimentation using immunoreactive insulin or pancreatectomized animals. This hypoglycemic effect could also be accounted for by some of the described properties of ouabain on carbohydrate metabolism *in vitro*. Ouabain decreases *in vitro* the breakdown of glycogen (1,3) probably through an inhibition of phospho-

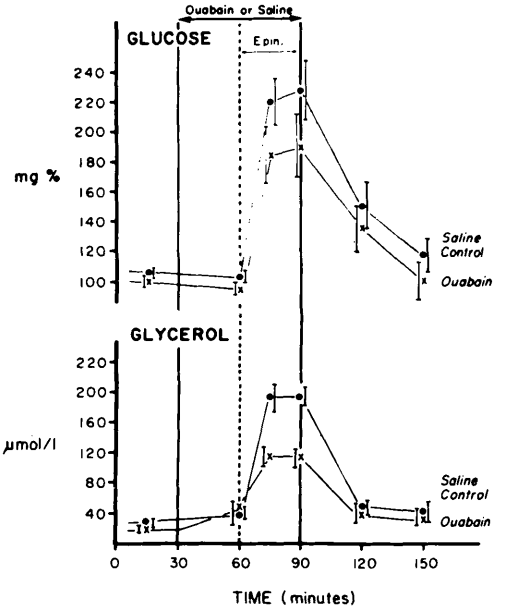

FIG. 2. Changes in blood glucose and glycerol concentrations due to epinephrine infusion in control (saline infusion) and ouabain-treated beagles. Means ± SE of 8 experiments are given. Although the glucose concentrations at the end of epinephrine infusion are not significantly different, the change from preinfusion levels in the ouabain-treated dogs is significantly lower ($p < 0.05$) than in the control dogs.

rylase. It also inhibits the glycolytic pathway at the phosphofructokinase rate-limiting step (7). If ouabain produced a decrease in liver glycogen breakdown (as it does in muscle glycogen) while glycolysis is inhibited and glucose uptake remains the same or increases, one could expect a diminution of the extracellular glucose pool. This would provide an explanation for a ouabain-induced hypoglycemia independent of insulin. A similar direct inhibitory effect of ouabain on lipolysis could account for the decrease in glycerol plasma concentration observed *in vivo*. In the fat pad, ouabain inhibits spontaneous as well as activated lipolysis. However, the release of glycerol produced by theophylline or cyclic 3′,5′-AMP is not inhibited by ouabain (8). These observations would indicate that ouabain exerts its inhibitory effect on the process which activates cyclic 3′,5′-AMP formation, namely adenylcyclase.

The interference of ouabain with the metabolic effects of epinephrine could be accounted for, in part, by Dengler (9) who showed that ouabain inhibits the uptake of catecholamines. However, it has also been shown *in vitro* that ouabain has metabolic effects, per se, independently of catecholamines and that these effects are antagonized by catecholamines. In the presence of ouabain, the glycogenolytic effect of epinephrine was significantly decreased in rat diaphragm (2, 3). In rat epididymal fat pads, ouabain inhibited glycerol release caused by epinephrine, but did not change the release induced by theophylline (2). In both rat diaphragm and adipose tissue, the effect of ouabain was simulated by omission of K^+ from the incubation media. The similarity of effects observed with ouabain or K^+ omission indicated that the inhibition of the metabolic effects of epinephrine in these two cases may be exerted through the same mechanism (10–13). The interaction of ouabain with the metabolic effects of epinephrine is not the only reported evidence of the antagonism of these two drugs. Regan *et al.* (14) have described an antagonistic action of epinephrine on the ionic and arrhythmic effects of strophanthidin. The interaction of ouabain with the metabolic effects of epinephrine is consistent with the observation (15) that ouabain decreases in the fat pad cyclic 3′,5′-AMP formation and, therefore, inhibits adenylcyclase. This same property was shown for insulin (16).

In addition to its cardiac effect, ouabain has a metabolic effect on other tissues. Further study is necessary to clarify the relationship between these effects in order to determine the mechanism of the therapeutic effect of this drug.

Summary. Ouabain (1.0 μg/kg per min) administered iv for 60 min in dogs caused a significant decrease in glucose and glycerol plasma concentration. The same dose of ouabain significantly inhibited the lipolytic and glycogenolytic effect of epinephrine. These metabolic changes caused by ouabain in intact animals might be explained either by a direct inhibitory effect on the adenyl cyclase system or by an indirect effect mediated through insulin.

Note: Experiments on pancreatectomized animals performed in this laboratory indicate that the hypoglycemic effect of ouabain is mediated through insulin.

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1. Clausen, T., Biochim. Biophys. Acta **120**, 361 (1966).

2. Triner, L., Kypson, J., and Nahas, G. G., Pharmacologist **9**, 236 (1967).

3. Kypson, J., Triner, L., Nahas, G. G., Pharmacologist **9**, 236 (1967).

4. Ho, R. J., Jeanrenaud, B., and Renold, A. E., Experientia **22**, 86 (1966).

5. Mosinger, B. and Kujalova, V., Biochim. Biophys. Acta **116**, 174 (1966).

6. Nahas, G. G. and Poyart, C., Am. J. Physiol. **212**, 765 (1967).

7. Kypson, J., Triner, L., and Nahas, G. G., J. Pharmacol. Exptl. Therap. 1968, in press.

8. Kypson, J., Triner, L., and Nahas, G. G., J. Pharmacol. Exptl. Therap. **159**, 8 (1968).

9. Dengler, H. J., "Pharmacology of Cholinergic and Adrenergic Transmission," p. 261. Macmillan (Pergamon), New York, 1965.

10. Schatzmann, J. H., Helv. Physiol. Pharmacol. Acta **11**, 346 (1953).

11. Judah, J. D. and Ahmed, K., Biol. Rev. Cambridge Phil. Soc. **39**, 160 (1964).

12. Skou, J. C., Prog. Biophys. Biophys. Chem. **14**, 133 (1964).

13. Skou, J. C., Physiol. Rev. **45**, 596 (1965).

14. Regan, T. J., London, B. L., Binak, K., and

Hellems, H. K., Circulation Res. 11, 17 (1962).

15. Ho, R. J., Jeanrenaud, B., Posternak, T. H., Renold, A. E., Biochim. Biophys. Acta 144, 74 (1967).

16. Butcher, R. W., Sneyd, J. G. T., Park, C. R., and Sutherland, E. W., Jr., J. Biol. Chem. 241, 1651 (1966).

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Induction of the Interferon System by Various Inducers*† (32924)

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Several laboratories (1,2) have shown that the development of interferon induced antiviral activity can be blocked by Dactinomycin (actinomycin D) and concluded that DNA-directed RNA synthesis was involved in this system. These findings also suggest that interferon does not itself inhibit virus replication but that it stimulates the production of new macromolecules by the cell—the proposed antiviral substance(s) (AVS) (1). The present report describes a comparative study of the interferon response and the development of resistance (proposed AVS?) in primary mouse embryo (ME) and African green monkey kidney (AGMK) cell cultures treated with virus, statolon, or interferon. The results indicate that a major portion of virus-induced interference in AGMK could be attributed to the interferon system, and that cellular resistance in ME and AGMK frequently develops before detectable interferon production.

Materials and Methods. The detailed procedures in this study were similar with those described in previous reports (3,4).

Inducers. The PR8 strain of influenza A virus, Semliki Forest virus (SLFV), rubella virus, Sindbis virus, monkey interferon or statolon (kindly supplied by Dr. W. Kleinschmidt, Eli Lilly Co.) (5) were used for induction of the interferon system in AGMK cell cultures. Statolon, polyoma, SV40, Sendai, and Newcastle disease (ND) viruses were employed in ME cell cultures.

Assay of interferon. The acidification method for destroying residual infectious virus in interferon preparations was previously described (3). Interferon was assayed in appropriate cell cultures by inhibition of vesicular stomatitis virus (VSV) yield. A unit of interferon was determined as the highest dilution of the sample which inhibited the final yield of VSV by 0.5 $\log_{10}$ during a single growth cycle (4). The titer of the NIH reference mouse interferon is 500 units/ml by this method. The AVS was estimated indirectly by determining the degree of cellular resistance to multiplication of VSV. Significant resistance was determined to begin at 0.5 $\log_{10}$ reduction of the final yield of VSV during a single growth cycle (6).

Assay of challenge viruses. All challenge viruses (SLFV, VSV, poliovirus type I - Mahoney strain, Sindbis virus or rubella virus) used in this experiment had at least a titer of $10^{7.0}$ plaque forming units (pfu) or 50% rubella interference doses (InD_{50}) per ml in order to insure a virus to cell multiplicity of greater than 10 for a single growth cycle infection. The yield of infectious virus harvested from the interference test was determined by plaque or infectivity titrations after treatment with the appropriate antiserum as described elsewhere (3,4,7).

Results. Dynamics of interferon response in

* Parts of these studies were conducted in the Department of Virus Research, Microbiological Associates, Inc., Bethesda, Maryland.

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