Effects of Dichloroisoproterenol on Blood Sugar and Plasma Free Fatty Acids.* (27182)

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The action of dichloroisoproterenol (DCI) as an adrenergic blocking agent has been extensively investigated. Powell and Slater(1) have observed that DCI antagonizes the action of epinephrine and isopropyl-arterenol on blood pressure of the cat, rabbit ileum and guinea pig tracheal chains. Moran and Perkins(2) have reported that DCI produced specific cardiac adrenergic blockade, preventing both the positive inotropic and phosphorylase-activating effects of epinephrine and sympathetic nerve stimulation(3).

In addition to serving as an adrenergic blocking agent, DCI has also been shown to possess certain sympathomimetic properties. Fleming and Hawkins(4) have concluded that the cardiac actions of this compound are more properly termed as competitive dualism rather than adrenergic blockade since doses of 0.01 to 3 mg have positive chronotropic and inotropic effects in the dog heart-lung preparation. Root(5) has demonstrated that in many species DCI elicits a hyperglycemic response similar to that produced by epinephrine, although it has been reported that DCI prevents release of 3′5′ cyclic adenylic acid(6) and effectively inhibits the hyperglycemic response to epinephrine in dogs(7).

The major metabolic effects of the adrenal medullary hormones are to produce an increase in blood glucose and plasma free fatty acids (FFA). We have recently reported(8) that DCI will effectively block the *in vitro* effects of epinephrine and isoproterenol on release of FFA by rat epididymal fat pads. The present study is concerned with the effects of DCI on blood glucose and plasma FFA levels in the anesthesized dog.

Methods. Chemical and physiological methods. Adult male and female mongrel dogs were anesthetized by intravenous administration of pentobarbital sodium (15 mg/kg) and small doses of pentobarbital given as required to maintain a constant depth of anesthesia. Animals were heparinized by injection of 1 cc heparin sodium intravenously, and 2 cc intramuscularly. A polyethylene catheter (PE 100) was inserted into the cephalic vein and connected to the sample tube of a technicon autoanalyzer programmed for glucose determination by the ferricyanide method(9). Blood was withdrawn at the rate of 0.3 ml/min. and blood sugar thus continuously recorded. A lag of 9 min. ensued between removal of the sample and response of the recording potentiometer.

Three ml samples of blood were withdrawn from the saphenous vein at specific time intervals, and FFA determined on 1 ml of plasma by the method of Dole(10).

Drugs. A stock solution of dl-isoproterenol hydrochloride (Isuprel), 2 mg/ml in isotonic saline, was freshly prepared for each experiment. Appropriate dilutions of this solution were made for the various doses tested. Dichloroisoproterenol (DCI), (β-hydroxy-N-isopropyl-3,4-dichlorophenethylamine) was similarly prepared in a stock solution of 20 mg/ml from which dilutions were made. Glucagon (3 mg/ml) was dissolved in 0.01 N HCl and subsequent dilutions made in saline.

In studies where DCI was the only drug administered, usually 3 or more successive doses of increasing strength were given to a single animal, allowing appropriate time for recovery between doses. When the effects of DCI on the response to isoproterenol or glucagon were determined, increasing doses of these drugs were administered during a control period and again following DCI. Usually 4 or more responses were recorded on a single animal.

In vitro *incubations.* Approximately 0.5 g of adipose tissue was incubated for 90 minutes in a Ringer-bicarbonate medium containing 4% bovine serum albumin. L-epinephrine was added to give a final concentra-

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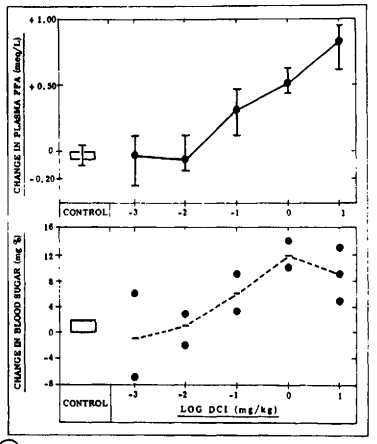

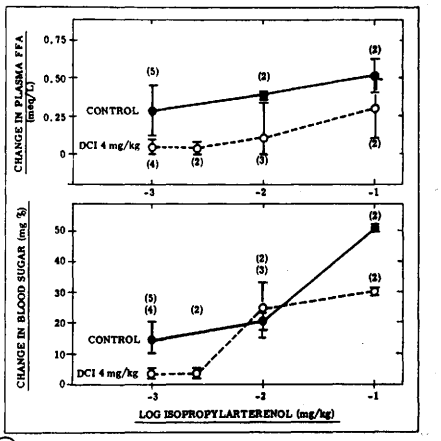

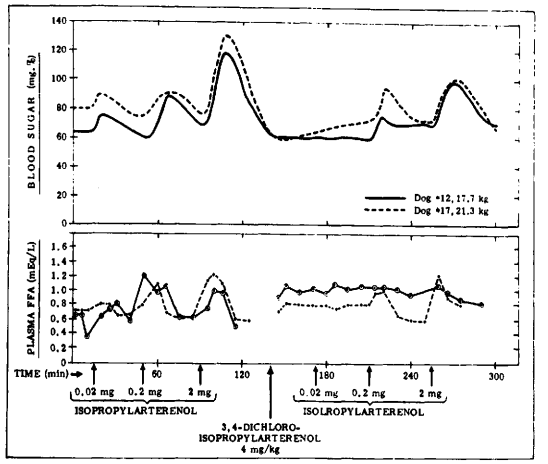

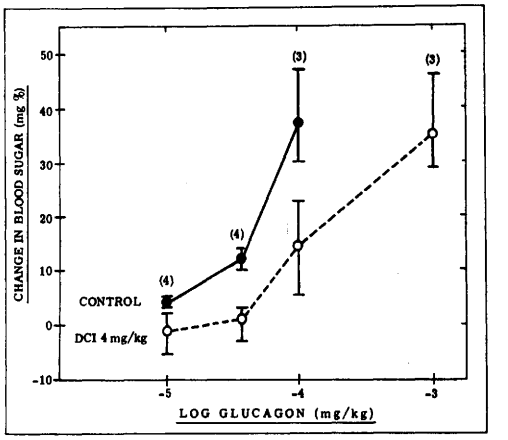

FIG. 1. Changes in plasma FFA (meq/l) and blood sugar (mg %) during a control period and following inj. of various doses of DCI are recorded. Values for plasma FFA represent the mean of 3 or more observations and ± stand. dev. are indicated. Changes in sugar represent means of 2 or more experiments. All values are indicated.

FIG. 2. Changes in plasma FFA (meq/l) and blood sugar (mg %) have been plotted in response to isopropylarterenol inj. during a control period (●——●) and after treatment with DCI, 4 mg/kg (○- - -○). No. of observations is indicated in parentheses, and ± stand. dev. is indicated where 3 or more observations were made.

FIG. 3. Changes in blood sugar and plasma free fatty acids (FFA) in response to isopropylarterenol before and after DCI. Upper curves represent continuous recording of blood sugar using the Technicon Autoanalyzer. In lower curves plasma FFA was determined on individual samples.

FIG. 4. Change in blood sugar (mg %) in response to glucagon inj. has been plotted for a control period (●——●) and after DCI, 4 mg/kg (○- - -○). No. of observations and stand. dev. is indicated.

tion of 10^{-4} M. and DCI when present was 10^{-3} M.

Results. The effects of DCI on plasma FFA and blood glucose of the anesthetized dog are summarized in Fig. 1. Doses of DCI from 0.001 mg/kg to 0.01 mg/kg were not effective in causing an increase in blood glucose or plasma FFA. However, doses of 0.1 mg/kg or greater caused a marked increase in plasma FFA and very little change in blood sugar. These results are in agreement with observations recently reported by Mayer, Moran and Fain(7). To test the effectiveness of DCI in blocking isopropylarterenol

TABLE I. FFA Release from Adipose Tissue *In Vitro* (All Values as μM FFA Released/g Tissue/90 Min.).

Tissue	No. observations	Control	Epi	DCI	DCI and Epi
Canine mesenteric	5	.3 ± .02*	4.1 ± .6	.5 ± .01	.2 ± .02
" subcutaneous	5	.8 ± .07	6.4 ± .8	1.4 ± .1	.8 ± .1
Rat epididymal	5	4.3 ± .4	20.0 ± 1.3	4.7 ± .6	4.9 ± .8

* Mean ± S.E.

(Fig. 2) and glucagon (Fig. 4) a dose of 4 mg/kg was selected.

Isopropylarterenol, in doses of 0.001 mg/kg and greater, causes an increase in plasma FFA and blood sugar in the anesthetized dog (Fig. 2). DCI, 4 mg/kg, reduces these effects of isopropylarterenol on FFA ($P<0.01$) and hyperglycemia ($P<0.05$) at doses of 0.001 mg/kg and 0.002 mg/kg, and may cause some reduction in response to isopropylarterenol up to 0.1 mg/kg.

Results of experiments on 2 dogs are given in Fig. 3. Continuous recording of glucose by the autoanalyzer makes possible accurate measurement of small changes in blood sugar. Maximum sugar responses to isopropylarterenol were observed in 5 to 10 minutes following injection. Plasma samples were collected for FFA determinations immediately before and at 5-minute intervals following injection of isopropylarterenol. Maximum response in FFA was observed within 5 to 15 minutes, and changes generally paralleled blood sugar. The responsiveness of FFA following DCI is difficult to interpret because of the marked elevation in plasma levels. One animal (Dog 12) gave no response to isopropylarterenol after DCI, while Dog 17 appears to have responded to higher doses. It is possible that in the case of Dog 12, DCI has produced a maximal elevation of plasma FFA and no further increase can occur.

DCI proved equally effective in reducing the hyperglycemic response to glucagon (Fig. 4). The hyperglycemic effect of 0.05 γ/kg of glucagon was completely blocked by 4 mg/kg of DCI ($P<0.01$) and the response to 0.1 γ/kg glucagon appears to have been markedly reduced. However, this dose of DCI does not preclude a hyperglycemic response to larger amounts of glucagon (2.0 γ/kg).

Since DCI does not stimulate FFA release from rat adipose tissue, *in vitro*(8), but does elicit a marked elevation of plasma FFA in the dog, the question arises as to whether this difference in FFA response to DCI is species variable, or due to differences between *in vitro* and *in vivo* systems. This was investigated by incubating canine adipose tissue *in vitro* under conditions identical to those used with rat tissue. These results are shown in Table I and values from rat adipose tissue are presented for comparison. It is obvious that the elevation of plasma FFA by DCI in the intact dog is an expression of its *in vivo* action rather than species difference.

Discussion. These results would indicate that DCI *in vivo* elevates the plasma FFA level of anesthetized dogs. This is in apparent contrast to the action of DCI on adipose tissue *in vitro*(8). However, Muhlbachova *et al.*(11) have found that DCI *in vivo* increased FFA levels in adipose tissue of the rat, a phenomenon known to precede FFA release(12). This may be in keeping with the weak sympathomimetic activity of DCI(2) which is often referred to as antagonistic or exhibiting "competitive dualism"(4) rather than true adrenergic blockade.

The effectiveness of DCI in reducing the hyperglycemic response to both isopropylarterenol and glucagon suggests that both compounds may react with an identical receptor site. It is well known that glucagon and epinephrine elicit hepatic glycogenolysis and hyperglycemia by an identical mechanism (13); however the initial reaction involving the hormone is not well defined.

The difference between *in vivo* and *in vitro* actions of DCI on FFA release is accentuated but not resolved by the present work. Adipose tissue from either the rat or the dog when challenged with DCI *in vitro* does not respond by increasing the rate of FFA release, and this compound actually inhibits the response of these tissues to epinephrine

and related substances. Our results indicate that DCI elevation of plasma FFA is dependent on its *in vivo* administration. *In vitro* studies suggest that DCI does not have an intrinsic lipolysis promoting property unlike epinephrine which stimulates FFA release *in vivo*(10,14) and *in vitro*(15,16). The evidence of Muhlbachova *et al.*(11) that DCI is mimetic to epinephrine, arterenol and isoproterenol does not preclude either the *in vivo* modification of DCI to a compound with greater lipolytic activity or release of FFA mobilizing factors within the organism. Another possible explanation is that DCI may interfere with utilization rather than the elaboration of plasma FFA. However, Armstrong *et al.*(17) have recently reported that DCI not only elevates plasma FFA levels, but also found that an increased rate of turnover is associated with elevation of plasma FFA levels.

Summary. 3,4,Dichloroisopropylarterenol (DCI) elevates the FFA level in anesthetized dogs. However, *in vitro* it does not cause FFA release from adipose tissue. Isoproterenol induces elevations of plasma FFA and glucose which are reduced by DCI (4 mg/kg). Glucagon hyperglycemia is also antagonized by DCI. DCI effects *in vivo* can be overridden by increasing the dose of isoproterenol or glucagon.

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Suppression of Experimental Allergic Encephalomyelitis by Stress.* (27183)

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Stress under certain conditions increases the non-specific resistance of the organism against injuries caused by various noxious agents(1-3). Stress influences certain immunologic phenomena as well, causing inhibition of active and passive anaphylaxis(4,5) and skin homograft rejection(6). The present report demonstrates that stress can suppress experimental allergic encephalomyelitis (EAE), a manifestation of delayed type hypersensitivity to nervous tissue.

It also occurred to us that the low incidence of EAE in certain species following administration of encephalitogenic emulsion by intraperitoneal route might be due to stressful effects of the inflammatory reaction to the inoculum in the peritoneal cavity. Waksman

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