Some Metabolic Actions of Orinase.* (23349)

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The precise mechanisms by which the hypoglycemic arylsulfonylureas exert their action(s) have not yet been fully elucidated. Demonstrations of alterations in several biological parameters have brought postulations of multiple sites of action(1,2,3,4). Among the suggested mechanisms have been: (a) stimulation of insulin secretion; (b) inhibition of glucose output by the liver; (c) inhibition of gluconeogenesis; (d) inhibition of glucagon secretion; (e) inhibition of insulinase. Analysis of published data indicates that no single mechanism is responsible for all observed effects and that several of the postulated mechanisms may be operative(5, 6,7). The evidence supporting a stimulation of insulin secretion is considerable(8,9,10, 11,12,13). However, failure to demonstrate any peripheral effects of insulin with arylsulfonylureas has not been reconciled with this interpretation(14,15,16).

The present studies were designed to derive additional information concerning metabolic responses to arylsulfonylureas. Attention was directed to its action on fasting glycemia and ketonemia in relation to known role of the liver in ketogenesis, and these responses were correlated with effect on liver glycogen. Observations were made on the influence of the drug on accumulation of glycogen in the dorsal interscapular fat pad of the rat because this tissue has been shown to be uniquely sensitive to the action of insulin(17,18,19). The results of these experiments are consistent with a mechanism of action mediated by insulin, but do not constitute proof of such a mechanism.

Methods. Male albino rats of the Vanderbilt strain, weighing 170-200 g were used. Orinase was administered in doses of 400 mg/kg in 0.5% $NaHCO_3$ by stomach tube in 4 cc volume. Control animals received equal volumes of the bicarbonate solution. Blood sugars were determined by the photometric method of Somogyi(20). Blood ketones were determined by the method of Werk *et al.*(21). For liver glycogen analyses, rats were anesthetized with nembutal and the entire liver removed. Livers, weighing 3-6 g, were divided. Two portions weighing 300-800 mg each were put in boiling 30% KOH. Another portion weighing 1-3 g was homogenized in cold 10% trichloroacetic acid in Waring blender. Glycogen determinations were done by the method of Carroll, Longley and Roe (22) using the anthrone reagent. Glycogen determinations of the dorsal interscapular fat pad were done as previously described(17), except for final analysis of glycogen(22).

Results. Tables I and II record the effect of Orinase on glycemia, ketonemia and liver glycogen of the 24 hour fasted rat. A marked depression of glycemia and ketonemia occurred within the first hour after Orinase administration. Blood sugar levels remained low and showed no significant rise by the sixth hour. The ketone levels demonstrated a significant rise by the third hour and had returned to pretreatment levels by the sixth hour. When a second dose of Orinase was administered at the sixth hour, the ketone levels could again be depressed without any further changes in blood sugar. Thus, in a group of 10 rats, blood ketone levels fell from 7.5 mg % to 2.6 mg % 2 hours after Orinase and then rose at 6 hours to 7.1 mg %. Two hours after a second dose of Orinase blood ketone values had again fallen to 2.8 mg %. The corresponding blood sugar values were 91, 40, 38 and 41 mg %.

Liver glycogen levels of the Orinase treated group were elevated over those of their re-

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TABLE I. Effect of Orinase on Blood Sugar and Ketone Body Levels in 24 Hour Fasted Rats.

Time, hr	Blood sugar, mg %			Blood ketones, mg %, as acetone		
	Control	Orinase	P	Control	Orinase	P
0	89 ± 1.8 (54)			7.1 ± .32 (28)		
1		44 ± .7 (11)			2.7 ± .02 (11)	
2	79 ± 2.3 (30)	39 ± 1.5 (49)	<.01	7.4 ± .48 (12)	2.9 ± .02 (36)	<.01
3		31 ± 4.0 (17)			4.3 ± .35 (18)	
5	76 ± 9.4 (16)	37 ± 1.9 (33)	"	7.9 ± .52 (16)	5.1 ± .33 (33)	"
6		43 ± 1.8 (18)			6.8 ± .37 (22)	
15	84 ± 4.3 (10)	78 ± 7.9 (19)	N.S.	7.6 ± .73 (12)	7.5 ± .48 (16)	N.S.

Results expressed as mean ± stand. error.
No. in parentheses refers to No. of animals used.

TABLE II. Effect of Orinase on Liver Glycogen (mg/100 g Liver) of 24 Hour Fasted Rats.

Time, hr	Control		Orinase		P
	KOH extraction	TCA extraction	KOH extraction	TCA extraction	
2	246 ± 39.0 (15)	172 ± 29.8 (14)	482 ± 46.0 (28)	349 ± 40.3 (28)	<.01
5	307 ± 47.2 (14)	206 ± 39.0 (14)	744 ± 91.4 (24)	487 ± 52.6 (24)	"
15	844 ±154.8 (7)	410 ± 93.3 (7)	777 ± 97.5 (12)	303 ± 33.3 (12)	>.5

No. in parentheses refers to No. of animals used.
Orinase—400 mg/kg p.o.
Difference between 2 hr control and 15 hr control—P >.02 <.05.

spective controls at both 2 and 5 hours. The level at 5 hours was significantly greater than that at 2 hours ($p<0.01$). At 15 hours the liver glycogen level of the Orinase treated group was not elevated over the 5 hour group or over its respective 15 hour control. The glycogen levels of the controls at 15 hours (39 hour fast) were higher than controls at 2 hours (26 hour fast), $P>0.02<0.05$. The glycogen changes were equally apparent in the KOH and TCA fractions.

Orinase had no effect on accumulation of glycogen in the dorsal fat pad of 24 hour fasted rats 2, 5, or 15 hours after its administration. To conserve space these data are not recorded. Since previous studies on glycogen deposition in the dorsal interscapular fat have shown that more pronounced glycogen elevations could be obtained by refeeding of glucose after a 48 hour fast(17,18,19), the experiment was repeated using these conditions. Rats were fasted 48 hours and divided into 3 groups. One group (16 rats) received Orinase at zero time, another group (12 rats) received subcutaneous injection of 1 unit of NPH insulin at zero time; and one group (12 rats) acted as controls. All these groups were tube fed 2 ml of 50% glucose at 2 hours, 1 ml of 50% glucose at 4 hours and were sacrificed at 6 hours. Both the insulin and Orinase treated groups had significantly lower blood sugars than the control group at 2, 4, and 6 hours. Insulin promoted deposition of glycogen in the dorsal fat pad (1085 ± 164.0 mg/100 g) while Orinase did not, (654 ± 78.5 mg/100 g), even though blood sugar depression was comparable in each case. The glycogen level in the control group was 637 ± 107.0 mg/100 g.

Mirsky has postulated that stimulation of insulin secretion is an early response to Orinase(23). He points out, however, that the response may be difficult to demonstrate in the fasted animal because fasting decreases the insulin content of the pancreas(6,24). We, therefore, reinvestigated the effect of Orinase on fat pad glycogen in the non-fasted rat. Because an immediate response was being sought, the animals were sacrificed at 3 hours after administration of the drug. One group received Orinase, one group received 1 unit of HGF free regular insulin subcutaneously, and the controls received bicarbonate. All groups received 2 ml of 50% glucose at start of experiment. The glucose was tube fed simultaneously with the Orinase and the bicarbonate to the respective groups. Table III shows that a highly significant increase in

TABLE III. Effect of Orinase and Insulin on the Dorsal Fat Pad Glycogen of the Rat.

Group	Blood glucose in mg % 0 hr	1 hr	3 hr	mg glycogen/100 g 3 hr	P
Glucose alone* (8)	131 ± 3.3	112 ± 5.7	90 ± 11.0	212 ± 29.5	—
" + Orinase† (8)	106 ± 2.9	64 ± 5.2	66 ± 2.5	662 ± 95.0	<.01
" + insulin‡ (6)	109 ± 2.6	55 ± 2.7	33 ± 3.3	1253 ± 226.0	"

* Glucose—2 ml of 50% glucose p.o. at start.
† Orinase—400 mg/kg p.o. at start plus glucose as above.
‡ Insulin—One unit H.G.P. free regular insulin inj. subcut. at start plus above glucose.
Results expressed as mean ± stand. error.
No. in parentheses refers to No. of animals.

adipose tissue glycogen content occurred in both the insulin and Orinase treated groups. This is a clear demonstration of a peripheral response to Orinase.

Discussion. Our experimental data are subject to a number of interpretations, but are not inconsistent with the immediate response to Orinase being mediated by stimulation of insulin secretion by the pancreas. They throw no new light on the mechanism of the prolonged action of the drug.

Although many data previously presented favor an action of the arylsulfonylureas on stimulation of insulin secretion, the failure to reproduce any of the usual peripheral actions of insulin with these drugs has barred acceptance of this interpretation(25). Mirsky(6), however, has cautioned that if an immediate secretion of insulin is only one of the actions of the drug, it might not be readily demonstrated if the insulin content of the pancreas is reduced when the experiment is carried out. Our study bears this out for it was impossible to demonstrate an increase in glycogen concentration of adipose tissue in the 24 or 48 hour fasted animal following Orinase administration, although insulin itself was effective. On the other hand, when the experiment was repeated in the fed animal, which would be expected to have adequate insulin stores, a prompt increase in adipose tissue glycogen content was noted. To our knowledge, only insulin has been found effective in increasing adipose tissue glycogen(17,18,19) and hence, until some other mechanism is described, this observation may be taken as strong presumptive evidence of an insulin action following Orinase. Krahl(26) was unable to detect an *in vitro* effect of the drug on adipose tissue metabolism.

The combination of hypoglycemia, a fall in fasting ketonemia and an accumulation of liver glycogen point to an hepatic action of Orinase. Other investigators have shown that the drug inhibits glucose output by the liver(27,28,29,30) and there is increasing evidence now that insulin has a similar action (31,32,33). The fall in blood sugar after Orinase is associated with a prompt but transient decline in ketonemia and a second dose of the drug after ketonemia has been reestablished immediately reduces ketone levels again. Insulin has an identical action‡ and it seems probable that this response reflects an action on hepatic ketogenesis(34). If one accepts the growing evidence, reviewed by Dunn *et al.*(33), that insulin does indeed have an action on the liver, the above data with Orinase are again consistent with an insulin-mediated action of the drug. Indeed, in view of the prompt antiketogenic effect of the drug and the absence of a demonstrable action on adipose tissue during fasting, it is conceivable that if insulin is secreted in response to Orinase ingestion, its major action during fasting may be on the liver rather than on the peripheral tissues. In evaluating the action of a drug which has the postulated action of stimulating insulin secretion it must be borne in mind that the response to insulin secreted into the portal vein and reaching the liver first may not be identical, qualitatively or quantitatively, with that of extracted insulin injected peripherally. This consideration may have bearing on some of the discrepancies in published reports concerning Orinase.

It should be emphasized that while our data

‡ To be published.

are compatible with the hypothesis that insulin secretion is stimulated, they certainly do not establish it. There are ample data to indicate that other mechanisms of action of the sulfonylurea drugs must also be operative (35,36,37,38). Further investigation is still necessary to establish the validity and significance of the various postulated actions of these agents.

Summary. 1. The fasted rat is responsive to the hypoglycemic effects of Orinase within one hour. Blood sugar is not restored until the fifteenth hour. 2. Orinase depresses the fasting ketosis of the rat. The effect is a rapid and transient one, but may be reobtained by a second dose of the drug. 3. Orinase causes a rise in liver glycogen of the fasting rat. 4. Orinase causes a deposition of glycogen in the interscapular fat pad of the fed, but not the fasted rat. 5. Similarities between the acute responses to Orinase and the action of insulin are discussed in relation to postulated hepatic and extra-hepatic actions of insulin.

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