PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 554–556 (1978)

Effects of Exogenous ATP on Glucoregulation *in Vivo*[1] (40244)

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Exogenous ATP *in vitro* prevents insulin stimulation of hexose transport and oxidation in isolated adipocytes (1, 2), presumably by phosphorylation of a membrane component and blockade of the transmission of signal transfer from the insulin receptor to the hexose carrier system. The purpose of the present study was to evaluate if exogenous ATP *in vivo* results in alterations of glucose regulation consistent with the insulin inhibition-membrane phosphorylation hypothesis. Specifically, ATP iv in rats was evaluated for effects on insulin lethality, hypoglycemic sensitivity, glucose tolerance, whole body glucose oxidation, and insulin sensitivity of excised epididymal fat pads.

Materials and methods. Overnight fasted male rats of the Holtzman strain (Holtzman Co., Madison, WI) weighing 300 ± 10 g were used, except for the epididymal fat pad studies in which 100 ± 10 g rats were chosen in order to provide optimal fat pad thickness. ATP was purchased from Sigma Chemical Company, St. Louis, MO as the Mg^{2+} salt and was prepared fresh daily by dissolving in 0.9% saline and adjusting the pH to 7.4. ATP was administered iv in doses of 10 μmoles per rat via the dorsal vein of the penis using light ether anesthesia. Crystalline insulin was purchased from Sigma Chemical Company and dissolved daily in 0.9% saline. Plasma glucose was determined using the Yellow Springs Model 23A Glucose Analyzer (Yellow Springs Instrument Co., OH). Whole body glucose oxidation was evaluated by the continuous recovery of expired $^{14}CO_2$ after the ip administration of 400 mg D-glucose containing 20 μCi of U-[^{14}C]D-glucose (3). Epididymal fat pad glucose oxidation was measured as described previously (4). Metabolic data were analyzed for statistical significance using Student's *t* test for unpaired comparisons. Lethality data were analyzed using the chi square test.

Results. Effect of ATP on insulin lethality. Male rats were treated with either saline or ATP iv concomitantly with insulin doses of 1, 2, 4 or 8 U, sc (Table I). ATP treatment was effective in reducing insulin induced seizure deaths at the 1 and 2 U insulin dosages; however, no protective effect was provided against the 4 and 8 U insulin dosages.

Effect of ATP on insulin hypoglycemia. Male rats were treated with either saline or ATP iv concomitantly with insulin doses of 0.05, 0.10, or 0.20 U sc (Table II). Plasma glucose depression in response to insulin was significantly blunted by ATP treatment.

Effect of ATP on glucose tolerance. Male rats were treated with either saline or ATP iv and 15 min later received 400 mg of D-glucose iv (Table III). The glucose disappearance half-time was increased by ATP, i.e. from 32.5 to 66.8 min.

Effect of ATP on in vivo glucose oxidation. Male rats were treated as specified in Table IV and whole body glucose oxidation measured. ATP depressed the oxidation of 400 mg glucose for 150 min after treatment (Group 1 vs 3) and also significantly blunted the insulin enhancement of glucose oxidation for 120 min after treatment (Group 2 vs 4).

TABLE I. EFFECT OF ATP (10 μMOLES IV) ON INSULIN LETHALITY.

Treatment group		Number of rats	% Lethality
ATP	Insulin (U sc)		
–	1	30	40
+	1	30	3*
–	2	30	67
+	2	30	33*
–	4	20	95
+	4	20	75
–	8	20	100
+	8	20	90

* $P < 0.05$ as compared to respective dose control group.

[1] Supported by USPHS Grant HL 08682.

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0037-9727/78/1584-0554$01.00/0

TABLE II. EFFECT OF ATP (10 μMOLES IV) ON INSULIN HYPOGLYCEMIA.

Experimental group	Number of rats	Insulin dose (U, sc)	Plasma glucose (mg/dl) at: 0	15′	30′	45′	60′
Saline	10	0.05	92 ± 2	85 ± 3	77 ± 2	78 ± 3	81 ± 4
	10	0.10	90 ± 3	75 ± 2	64 ± 2	60 ± 3	60 ± 2
	10	0.20	94 ± 3	70 ± 2	60 ± 3	54 ± 2	49 ± 3
ATP	10	0.05	90 ± 3	92 ± 4	90 ± 3*	89 ± 2*	88 ± 3*
	10	0.10	91 ± 2	89 ± 3*	84 ± 4*	78 ± 2*	79 ± 3*
	10	0.20	93 ± 3	87 ± 4*	85 ± 3*	80 ± 2*	72 ± 4*

* $P > 0.05$ as compared to respective saline control dose-time group. Means ± SEM.

TABLE III. EFFECT OF ATP (10 μMOLES IV) ON GLUCOSE TOLERANCE.[a]

Experimental group	Number of rats	Glucose Disappearance half-time (mean ± SEM)
Saline control	22	32.5 ± 2.5 min
ATP	17	66.8 ± 3.5 min*

* $P < 0.05$.

[a] 400 mg D-glucose iv 15 min after treatment. Means ± SEM.

Effect of ATP on glucose oxidation in adipose tissue. Male rats were treated with either saline or ATP iv and epidiymal fat pads were removed at 15–30 min thereafter (Table V). Basal glucose oxidation was not affected (Groups 1 vs 3) but the insulin stimulated response was significantly depressed by ATP treatment (Groups 2 vs 4).

Discussion. Using *in vitro* test systems, exogenous ATP has been reported to decrease insulin stimulated hexose transport in a manner consistent with phosphorylation of a membrane transport component (1, 2). The current study addressed the question: will exogenous ATP administered *in vivo* alter *in vivo* glucoregulation and *in vitro* responses to insulin? The data presented indicate that ATP given iv, *in vivo* induces a glucoregulatory state consistent with inhibited insulin responsiveness, i.e. ATP decreased insulin convulsive seizure lethality, blunted insulin induced hypoglycemia, decreased the disappearance rate of iv glucose, and impaired whole body glucose oxidation of 400 mg glucose lead, with or without concomitant insulin treatment. Furthermore, ATP *in vivo* depressed the glucose oxidation in epididymal fat pads excised and exposed to insulin *in vitro*.

These *in vivo* and *in vitro* findings are consistent with the previous reports on ATP effects *in vitro* (1, 2). The data offer promise that the glucoregulatory status *in vivo* is amenable to alteration by exogenous phosphorylating nucleotides. Other modes of ATP action, as for example by inducing hypotension and causing secondary epinephrine release, are potentially applicable to inhibition of insulin action. Lastly, the reported anti-insulin effects of ATP *in vivo* are consistent with our recent finding that exogenous ATP can ameliorate the defects in glucoregulation occurring in endotoxin shock (4).

Summary. The effect of exogenous ATP

TABLE IV. EFFECT OF ATP (10 μMOLES) ON *in vivo* GLUCOSE OXIDATION.

Experimental group iv	sc	No. of rats	% Glucose to CO_2 (min) 15	30	45	60	90	120	150	180	210	240
1. Saline	Saline	8	0.15 ± 0.02	1.00 ± .16	2.93 ± .36	5.39 ± .58	10.99 ± 0.81	16.50 ± 0.89	21.48 ± 0.91	26.19 ± 1.02	30.31 ± 1.10	34.10 ± 1.16
2. Saline	Insulin (.10U)	9	0.19 ± 0.03	1.51 ± .19	4.00 ± 0.36	7.46 ± 0.48	16.05 ± 0.88	24.38 ± 1.28	31.04 ± 1.53	35.85 ± 1.69	39.28 ± 1.69	41.14 ± 1.96
3. ATP	Saline	11	0.08 ± 0.01	0.62 ± 0.06	1.72 ± 0.13	3.32 ± 0.21	7.53 ± 0.41	12.29 ± 0.65	17.18 ± 0.85	25.34 ± 3.72	28.97 ± 1.08	35.52 ± 5.94
4. ATP	Insulin (.10U)	8	0.13 ± 0.02	1.09 ± 0.14	3.18 ± 0.36	6.30 ± 0.63	14.00 ± 1.16	20.02 ± 1.79	28.51 ± 2.06	37.42 ± 5.32	37.39 ± 2.37	40.20 ± 2.39
P values <0.05: 1 vs 3			+	+	+	+	+	+	+	−	−	−
P values <0.05: 2 vs 4			+	+	+	+	+	+	−	−	−	−
Means ± SEM												

TABLE V. EFFECT OF ATP (10 μMOLES IV) *in vivo* ON GLUCOSE OXIDATION AND INSULIN RESPONSE OF ISOLATED EPIDIDYMAL FAT PADS.

Experimental group	Insulin (.25 mU/ml)	No. of preps.	Glucose oxidation dpm-$^{14}CO_2$/g/60 min
1. Saline	–	10	42,852 ± 2488
2. Saline	+	10	101,888 ± 7654
3. ATP	–	12	44,102 ± 1656*
4. ATP	+	12	68,432 ± 4211*

* *P* Values <0.05: 1 vs 2; 3 vs 4; 2 vs 4.
Means ± SEM.

treatment (10 μmoles iv) on glucoregulation was evaluated in male Holtzman rats. ATP treatment decreased lethality of insulin in doses of 1 and 2 U sc, blunted the hypoglycemic response to 0.05, 0.10 and 0.20 U insulin sc, increased the iv glucose disappearance rate, depressed the *in vivo* oxidation of 400 mg glucose, and inhibited the insulin response of epididymal fat pads to insulin *in vitro*. The data are consistent with the Chang-Cuatrecasas hypothesis that exogenous ATP inhibits insulin-mediated effects on glucose metabolism via phosphorylation of a membrane component.

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Received January 9, 1978. P.S.E.B.M. 1978, Vol. 158.