

Urethane-induced hyperglycemia¹

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ABSTRACT

AIM: To study the effects of urethane, at anesthetic dose, on the blood glucose levels in normal rats and hyperglycemic rats, and its effects on the hypoglycemic action of exogenous insulin in alloxan-treated rats.

METHODS: Blood glucose concentration was measured with the glucose oxidase method.

RESULTS: Urethane at anesthetic dose $1.5 \text{ g} \cdot \text{kg}^{-1}$ increased the blood glucose levels in fasting (to $2.6 \pm 0.3 \text{ g} \cdot \text{L}^{-1}$, $P < 0.01$) or glucose-loaded (to $3.9 \pm 0.4 \text{ g} \cdot \text{L}^{-1}$, $P < 0.01$) rats. It did not modify the hyperglycemia induced by epinephrine (normal islet β -cells) or alloxan (impaired islet β -cells).

In the rats treated with alloxan, blood glucose level decreased to $1.8 \pm 0.7 \text{ g} \cdot \text{L}^{-1}$ at 200 min after administration of insulin from control level of $7.0 \pm 2.3 \text{ g} \cdot \text{L}^{-1}$, but the hypoglycemic action of exogenous insulin was abolished by urethane.

CONCLUSION: Hyperglycemic action of urethane was due to its inhibiting effect on the hypoglycemic effect of insulin, except for its known mechanism of increased sympathetic release.

INTRODUCTION

Urethane has been widely used as a long-term anesthetic for small experimental animals^[1,2], because it does not influence the central nervous system (CNS) involved in the control of cardiovascular functions as compared with other anesthetics^[3], and the peripheral stimuli are still able to activate CNS and produce reflex changes in autonomic functions in urethane-anesthetized animals^[4]. In some diabetic experiments, animals were

anaesthetized by barbiturate^[5,6] or urethane^[7] to avoid the influence (pain, local damage and stress) of collecting blood samples on the blood glucose level of the animals. It was reported, however, that urethane increased blood glucose levels in rats and rabbits^[8-13], but barbiturate at anesthetic dose did not induce hyperglycemia^[10,14,15]. Urethane-induced hyperglycemia in the rat was prevented by hypophysectomy, bilateral adrenalectomy^[10,14,15], α_2 receptor antagonist yohimbine^[8,11] or reserpine^[13]. Reinfr^[13] speculated that increased sympathetic activity led to enhanced catecholamine secretion from the adrenal medulla, which, in turn, regulated the concentration of blood glucose. On the other hand, there were some studies indicating that urethane-induced hyperglycemia in the cat was independent of liver glycogen levels and epinephrine release^[16], and significant increase of the blood glucose levels by urethane was observed in fasting rats but not in fed rats^[9]. The present study was designed to investigate the effects of urethane at anesthetic dose on blood glucose level in fasting or glucose loaded rats, in alloxan-induced hyperglycemic rats (impaired islet β -cells) or epinephrine-induced hyperglycemic rats (normal islet β -cells). Effects of urethane on the hypoglycemic action by exogenous insulin were also observed in alloxan-treated rats.

MATERIALS AND METHODS

Chemicals Urethane (Sigma Chemical Co) and epinephrine hydrochloride (Wuhan Pharmaceutical Corporation) were dissolved in normal saline at concentrations of $250 \text{ g} \cdot \text{L}^{-1}$ and $0.05 \text{ g} \cdot \text{L}^{-1}$, respectively. Blood glucose kit was the product of Shijiazhuang Reagent Factory. Insulin (Chinese Pharmaceutical Inspection Institute) was dissolved in normal saline at concentration of $0.05 \text{ g} \cdot \text{L}^{-1}$.

Rats Sprague-Dawley rats, δ , weighing (210 ± 30) g, were provided by Experimental Animal Center of Hebei Medical University (Grade II, Certificate No 035). All animals were fed for a week in laboratory before

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experiment, and fasted 24 h before blood samples were collected from orbital vein.

Blood samples and blood glucose measurement

Blood samples were collected from the orbital vein using heparin-containing glass capillaries. Blood was centrifuged to separate the plasma, and glucose was measured immediately after separation using glucose oxidase method^[17].

Fasting rat Sixteen rats were divided into two groups, one was treated with urethane $0.75 \text{ g} \cdot \text{kg}^{-1}$ ip and $0.75 \text{ g} \cdot \text{kg}^{-1}$ sc^[18] (the same in the following experiments). The other group was treated with saline as control. Blood samples were collected before and 20, 50, 80, 140, and 200 min after injection of urethane or saline.

Glucose tolerance test Sixteen rats were divided into two groups. They were treated with urethane and saline respectively, and then treated with ig glucose $5 \text{ g} \cdot \text{kg}^{-1}$ immediately. Blood samples were collected before and 20 min after urethane, and 30, 60, 90, and 120 min after loading glucose.

Epinephrine-induced hyperglycemic rats Sixteen rats were divided into two groups, which were treated with urethane and saline respectively and both groups were treated with sc epinephrine at $0.2 \text{ mg} \cdot \text{kg}^{-1}$ simultaneously. Blood samples were collected before and 20, 50, 80, 140, and 200 min after the treatments.

Alloxan-induced hyperglycemic rat Rats were injected with alloxan $0.25 \text{ g} \cdot \text{kg}^{-1}$ ip, and the blood samples were collected in 48 h. Rats whose blood glucose levels were 5 times higher than those before treatment were used as hyperglycemic rats. Sixteen hyperglycemic rats

were divided into two groups, to whom urethane and saline were injected respectively. Another sixteen hyperglycemic rats were divided into two groups to observe the effects of urethane and saline on the hypoglycemic action of exogenous insulin. Exogenous insulin was given at $4.5 \text{ IU} \cdot \text{kg}^{-1}$ sc immediately after urethane and saline. Blood samples were collected before and 20, 50, 80, 140, and 200 min after administration of urethane or both urethane and exogenous insulin.

Statistical methods Data were expressed as $\bar{x} \pm s$, and two way ANOVA was used to evaluate the significance between the date of control and treatment groups. If the *F* statistic value was significant, we compared the individual datum with its respective control value using *t*-test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Effects of urethane on the fasting rat Blood glucose levels did not change during the experimental period in the control animal. However, urethane caused an obvious increase in blood glucose level, and the blood glucose increased 50 min after administration of urethane $1.5 \text{ g} \cdot \text{kg}^{-1}$ ($P < 0.01$, Tab 1). The peak level of blood glucose in the rat treated with urethane was 2.1 times higher than that in the control animal ($P < 0.01$, Tab 1).

Effects of urethane on the glucose-loading rats Blood glucose level increased 30 min after ig glucose in the control group, and its peak time was 2 h (Tab 1).

Urethane $1.5 \text{ g} \cdot \text{kg}^{-1}$ ($P < 0.01$) increased the

Tab 1. Effects of urethane on the blood glucose level in the rat. $n = 8$ rats. $\bar{x} \pm s$. ^a $P > 0.05$, ^c $P < 0.01$ vs control.

Groups	Dosage/ $\text{g} \cdot \text{kg}^{-1}$	Blood glucose/ $\text{g} \cdot \text{L}^{-1}$					
		0	20	50	80	140	200 (min)
Fasting rat							
Control	-	0.80 ± 0.06	0.82 ± 0.06	0.76 ± 0.04	0.80 ± 0.06	0.85 ± 0.06	0.78 ± 0.05
Urethane	1.5	0.79 ± 0.07^a	0.89 ± 0.11^a	1.5 ± 0.4^c	2.3 ± 0.3^c	2.6 ± 0.3^c	2.23 ± 0.20^c
Glucose-loaded rat							
Control	-	0.81 ± 0.06	0.84 ± 0.11	1.76 ± 0.28	2.33 ± 0.22	2.44 ± 0.26	2.1 ± 0.3
Urethane	1.5	0.82 ± 0.06^a	0.89 ± 0.11^a	3.9 ± 0.4^c	4.3 ± 0.6^c	3.9 ± 0.4^c	3.52 ± 0.25^c
Hyperglycemic rat induced by epinephrine							
Control	-	0.81 ± 0.08	1.21 ± 0.15	1.84 ± 0.27	2.0 ± 0.4	1.7 ± 0.4	1.45 ± 0.28
Urethane	1.5	0.84 ± 0.08^a	1.32 ± 0.20^a	1.84 ± 0.09^a	2.2 ± 1.0^a	1.9 ± 0.9^a	2.0 ± 1.0^a
Hyperglycemic rat induced by alloxan							
Control	-	9.2 ± 1.6	9.4 ± 1.5	10.3 ± 1.8	9.5 ± 1.6	10.3 ± 1.4	10.1 ± 1.9
Urethane	1.5	9.5 ± 2.5^a	10.3 ± 3.2^a	10.1 ± 2.4^a	9.9 ± 2.6^a	12.4 ± 3.4^a	12.0 ± 1.5^a

levels of blood glucose 0.5 , 1 , 2 , and 3 h after ig glucose by 125 % , 83 % , 60 % and 68 % , respectively , compared with the control group (Tab 1).

Effects of urethane on epinephrine-induced hyperglycemia The levels of blood glucose were increased 20 , 50 , 80 , 140 , and 200 min after administration of epinephrine , compared with the levels before epinephrine ($P < 0.01$) in the control group (Tab 1). Urethane treatment did not affect the hyperglycemic action of sc epinephrine ($P > 0.05$, Tab 1).

Effects of urethane on alloxan-induced hyperglycemia The levels of blood glucose increased to (9.34 ± 2.05) mg/L ($n = 16$) 48 h after ip alloxan , which were 9 times higher than the blood glucose levels before alloxan ip. The hyperglycemic levels were stable during the experimental period , and were not affected by urethane in comparison with control group ($P > 0.05$, Tab 1).

Effects of urethane on the hypoglycemic action of exogenous insulin in alloxan-treated rat The levels of blood glucose gradually decreased after administration of exogenous insulin $4.5 \text{ IU} \cdot \text{kg}^{-1}$ in alloxan-treated rats. The hyperglycemia induced by alloxan decreased 20 , 50 , 80 , 140 , and 200 min after insulin ($P < 0.01$, Tab 2) , and the blood glucose level decreased to (1.8 ± 0.7) $\text{g} \cdot \text{L}^{-1}$ at 200 min after administration of insulin from pretreatment level of (7.0 ± 2.3) $\text{g} \cdot \text{L}^{-1}$. Urethane suppressed the hypoglycemic action of exogenous insulin. The level of blood glucose decreased to (4.7 ± 2.6) $\text{g} \cdot \text{L}^{-1}$ at 200 min after administration of insulin , and which was not different from the pretreatment with insulin in urethane-treated rats ($P > 0.05$).

DISCUSSION

Urethane at anesthetic dose increased the blood glucose levels in glucose loading rats , however it did not modify the hyperglycemia induced by epinephrine or alloxan. Besides , we observed that hypoglycemic action of exogenous insulin was obviously inhibited by urethane in

the rat treated with alloxan. These results demonstrated that urethane-induced hyperglycemia was at least partly due to its inhibitory effect on the hypoglycemic effect of insulin , except for its known mechanism of increasing sympathetic discharge^[8 , 11 , 13].

In the present study , urethane increased blood glucose concentration in fasting rats , but it did not affect that animals treated with phenobarbital sodium at anesthetic dose of $0.1 \text{ g} \cdot \text{kg}^{-1}$ (data not shown). We reconfirmed that anesthetic condition itself did not affect blood glucose levels obviously in the rat^[10 , 13 , 14]. Urethane further increased the blood glucose concentration in glucose loaded rats , which might be explained with both mechanisms of an enhanced release of epinephrine^[8 , 11 , 13] and an inhibitory action on the effects of endogenous insulin.

It is well-known that epinephrine produces hyperglycemia via both stimulation of hepatic gluconeogenesis and inhibition of insulin release from the islets of Langerhans^[19-21]. An epinephrine-induced hyperglycemia was observed in this experiment , however , urethane did not further increase the levels of hyperglycemia induced by epinephrine. Marley and Paton^[22] reported that urethane releases catecholamines within a limited and physiological range , and the exogenous epinephrine decreases the secretion of insulin^[19-21]. These reports could provide a rational explanation for the ineffectiveness of urethane in the rat treated by epinephrine. Islet β -cells were impaired by alloxan and had lost their function of secretion insulin mostly , which resulted in hyperglycemia^[23]. The present data showed that alloxan produced a much higher increase in blood glucose levels , in comparison with that in urethane-treated normal rats. Therefore , urethane treatment could not cause a further rise in the blood glucose levels in alloxan-treated rats.

The main conclusion that urethane-induced hyperglycemia was at least partly due to the inhibition of the hypoglycemic effect of insulin by urethane , was also supported indirectly by two previous observations.

Tab 2. Effects of urethane on the hypoglycemic action of exogenous insulin ($4.5 \text{ IU} \cdot \text{kg}^{-1}$, sc) in the hyperglycemic rat induced by alloxan. $n = 8$ rat. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs before insulin.

Groups	Dosage/ $\text{g} \cdot \text{kg}^{-1}$	Blood glucose/ $\text{g} \cdot \text{L}^{-1}$					
		0	20	50	80	140	200 (min)
Insulin	-	7.0 ± 2.3	4.9 ± 1.4^b	4.1 ± 1.4^b	3.7 ± 1.2^c	3.13 ± 0.11^c	1.8 ± 0.7^c
Insulin + urethane	1.5	6.9 ± 2.4	7.0 ± 2.0^a	6.4 ± 2.3^a	6.1 ± 2.9^a	5.9 ± 3.0^a	4.7 ± 2.6^a

Sollman^[16] reported that urethane-induced hyperglycemia in the cat was independent of epinephrine release^[16], and Sanchez Pozo *et al*^[9] showed that a decrease in the consumption of glucose and an increase in lipolysis occurred in urethane-anesthesia rats. Further experiments are needed to investigate which steps are involved in the inhibitory effects of urethane on the hypoglycemic action of insulin with respect to glucose metabolism. In any case, the effects of urethane anesthesia on rats should be considered in physiological or pharmacological studies, particularly in metabolic studies.

REFERENCES

- 1 Katafuchi T, Koizumi K. Fastigial inputs to paraventricular neurosecretory neurons studied by extra and intracellular recordings in rats. *J Physiol (London)* 1990 ; 421 : 535 - 51.
- 2 Conte B, Maggi A, Parlani M, Lopez G, Manzini S, Giachetti A. Simultaneous recordings of vesical and urethral pressure in urethane-anesthetized rats : effects of neuromuscular blocking agents on the activity of the external urethral sphincter. *J Pharmacol Methods* 1991 ; 26 : 161.
- 3 Lalley PM. Inhibition of depressor cardiovascular reflexes by a derivative of γ -aminobutyric acid (GABA) and by general anesthetics with suspected GABA-mimetic effects. *J Pharmacol Exp Ther* 1980 ; 215 : 418 - 25.
- 4 Cross BA, Silver IA. Unit activity in the hypothalamus and the sympathetic response to hypoxia and hypercapnia. *Exp Neurol* 1963 ; 7 : 375 - 95.
- 5 Maitra SR, Krikhely M, Dulchavsky SA, Geller ER, Kreis DJ. Beneficial effects of diltiazem in hemorrhagic shock. *Circulatory Shock* 1991 ; 33 : 121 - 5.
- 6 Fehmann HC, Stockmann F, Haverich R, Creutzfeldt W. Diltiazem inhibition of glucose-induced insulin secretion from the isolated perfused rat pancreas. *Eur J Pharmacol* 1988 ; 156 : 415 - 7.
- 7 Takahara J, Yunoki S, Yamauchi J, Yakushiji W, Hosogi H, Ofuji T. Effect of glucagon on growth hormone secretion in rats. *Horm Metab Res* 1978 ; 10 : 227 - 30.
- 8 Aisaka K, Kihara T, Koike M, Kuroki M, Ishihara T. Effect of yohimbine on urethane-induced hyperglycemia in rats. *Jpn J Pharmacol* 1989 ; 49 : 523 - 7.
- 9 Sanchez-Pozo A, Alados JC, Sanchez-Medina F. Metabolic changes induced by urethane-anesthesia in rats. *General Pharmacol* 1988 ; 19 : 281 - 4.
- 10 Kansal PC, Buse MG. The effect of adrenergic blocking agents on plasma insulin and blood glucose during urethane or epinephrine-induced hyperglycemia. *Metabolism* 1967 ; 16 : 548 - 56.
- 11 Takeuchi K, Niida H, Ohuchi T, Okabe S. Influences of urethane anesthesia on indomethacin-induced gastric mucosal lesions in rats. Relation to blood glucose levels. *Digestive Diseases & Sciences* 1994 ; 39 : 2536 - 42.
- 12 Spriggs TL. The effects of anesthesia induced by urethane or phenobarbitone upon the distribution of peripheral catecholamines in the rat. *Br J Pharmacol* 1965 ; 24 : 752 - 8.
- 13 Reinfirt H. Urethane hyperglycemia and hypothalamic activation. *Nature* 1964 ; 204 : 889 - 91.
- 14 Hinton BT. Hyperglycemia in urethane-anesthetized rats : involvement of the adrenal gland. *Lab Anim Sci* 1982 ; 32 : 251 - 2.
- 15 Krzemien K, Lipowska-Zabawska L. Urethane-induced hyperglycemia in rats. *Acta Physiol Pol* 1976 ; 27 : 369 - 76.
- 16 Sollman TA. *Manual of Pharmacology*. 8th ed. Philadelphia : Saunders ; 1957.
- 17 Zhou XK. Blood or plasma glucose assay : application of glucose oxidase method. *Chin J Med Lab Tech* 1982 ; 5 : 56 - 60.
- 18 Mcleod LJ. *Pharmacological experiments on intact preparations*. 1st ed. Edinburgh London and New York : Churchill Livingstone ; 1970. p63.
- 19 Nakadate T, Nakaki T, Muraki T, Kato R. Adrenergic regulation of blood glucose levels : possible involvement of postsynaptic alpha-2 type adrenergic receptors regulating insulin release. *J Pharmacol Exp Ther* 1980 ; 215 : 226 - 30.
- 20 Nakadate T, Nakaki T, Muraki T, Kato R. Regulation of plasma insulin level by α_2 - adrenergic receptors. *Eur J Pharmacol* 1980 ; 65 : 421 - 4.
- 21 Nakaki T, Nakadate T, Ishii K, Kato R. Postsynaptic alpha-2 adrenergic receptors in isolated rat islets of Langerhans : Inhibition of insulin release and cyclic 3', 5'-adenosine monophosphate accumulation. *J Pharmacol Exp Ther* 1980 ; 216 : 607 - 12.
- 22 Marley E, Paton WDM. The output of sympathetic amines from the cat adrenal gland in response to splanchnic nerve activity. *J Physiol (London)* 1961 ; 155 : 1 - 27.
- 23 Heikila RE, Cabbat FS. The prevention of alloxan-induced diabetes by amygdalin. *Life Sci* 1980 ; 27 : 659 - 62.

乌拉坦诱导的高血糖反应¹

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关键词 乌拉坦; 血糖; 胰岛素; 肾上腺素; 四氧嘧
啶; 禁食

目的: 观察麻醉剂量的乌拉坦对空腹大鼠、葡萄糖
负荷大鼠, 肾上腺素或四氧嘧啶诱发高血糖大鼠血

糖水平的影响, 并探讨其对外源性胰岛素降血糖作
用的影响. 方法: 葡萄糖氧化酶法测定血糖含量.
结果: 麻醉剂量 $1.5 \text{ g} \cdot \text{kg}^{-1}$ 乌拉坦(sc, ip 各半)显著
升高空腹大鼠和葡萄糖负荷大鼠的血糖水平, 但对
肾上腺素(胰岛功能正常)或四氧嘧啶(胰岛功能受
损)诱发的高血糖大鼠的血糖水平无明显影响. 在
四氧嘧啶诱发的高血糖大鼠, 乌拉坦显著对抗外源
性胰岛素的降血糖作用. 结论: 乌拉坦升高血糖的
作用除与已知的释放肾上腺素有关外, 抑制胰岛素的
降血糖作用也是其升高血糖的机制之一.

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