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EFFECTS OF (−)-(R)-1-(*p*-HYDROXYPHENYL)-2-[(3,4-DIMETHOXYPHENETHYL)AMINO]ETHANOL (TA-064), A NEW CARDIOTONIC AGENT, ON CIRCULATING PARAMETERS OF CARBOHYDRATE AND LIPID METABOLISM IN THE RAT

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Abstract—Effects of the new cardiotonic and selective β_1-adrenergic agonist TA-064, (−)-(R)-1-(*p*-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol, on circulating concentrations of glucose, lactate, free fatty acids (FFA), glycerol, cyclic AMP and the pancreatic hormones insulin (IRI) and glucagon (IRG) were examined in rats. TA-064, administered orally or intraperitoneally at the dose of 10 mg/kg (*ca.* 50 times the therapeutic dose) or higher, caused a slight transient rise followed by a persistent lowering of blood glucose concentrations, but it did not affect blood lactate levels at all. The same treatment with TA-064 elevated the concentrations of blood FFA, glycerol and plasma IRI and IRG. These changes induced by TA-064 were inhibited by pretreatment with propranolol (a non-selective β-adrenergic antagonist) and practolol (a selective β_1-adrenergic antagonist). The non-selective β-adrenergic agonist isoproterenol and the selective β_2-adrenergic agonist terbutaline elevated both blood glucose and lactate when administered intraperitoneally. They also brought about sustained rises in blood glycerol and plasma IRI, but only transiently increased the plasma IRG level. The cardiotonic agent prenalterol, claimed to be a selective β_1-agonist, elevated blood glucose, lactate, and glycerol only slightly, and plasma IRI significantly, but it had no effect on plasma IRG. The cardiotonic agents dobutamine and amrinone also elevated blood glucose. Thus, TA-064 is unique among the β-adrenergic and other cardiotonic agents in that it produces sustained hypoglycemia while it has no lactacidemic effect. Since this hypoglycemic action of TA-064 was always preceded by a rise in plasma IRI and abolished in streptozotocin-diabetic rats, we conclude that increased secretion of pancreatic insulin and the lack of hyperglycemic action are responsible for the hypoglycemia by high doses of TA-064.

Stimulation of β-adrenergic receptors is known to elevate blood concentrations of glucose, lactate, glycerol and free fatty acids (FFA) [1], and increase the secretion of the pancreatic hormones insulin and glucagon [2]. Beta-receptors have been divided into two subtypes, β_1 and β_2, since the proposal by Lands *et al.* [3, 4]. Investigations using some selective β_2-agonists and β_1- and β_2-antagonists have shown that, in rats, stimulation of β_2-receptors is responsible for the elevation of blood glucose and lactate levels [5, 6], while stimulation of β_1-receptors results in elevations of the concentrations of blood FFA and glycerol [3, 4] and plasma insulin (IRI) [7, 8].

Reports of effects of β_1-adrenergic agonists on carbohydrate and lipid metabolism have been scarce, probably because few selective β_1-adrenergic agonists with minimal β_2- and α-adrenergic actions are available. Prenalterol, claimed to be a selective β_1-adrenergic agonist [9], was reported to increase the levels of plasma glycerol, FFA and insulin, but not to affect the levels of plasma glucose and lactate in man [10]. Similar observations have been reported on another selective β_1-agonist, tazolol [11].

Nagao *et al.* have reported a new cardiotonic compound, (−)-(R)-1-(*p*-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol (TA-064), which exerts a strongly positive inotropic action and possesses very weak vasodilating and no α-agonistic activities [12], and this inotropic effect was depressed by pretreatment with the selective β_1-adrenergic antagonist practolol [12].

The purpose of the present investigation was to examine effects of this new β_1-adrenergic agonist TA-064 on circulating parameters of carbohydrate and lipid metabolism in rats in comparison with other known β-adrenergic agents. Such studies are expected to help define metabolic effects of β_1-adrenergic stimulation. We found that, in contrast with non-selective β- and selective β_2-adrenergic agonists which invariably elevate both blood glucose and lactate, TA-064 produced sustained hypoglycemia after a slight and short-lived rise in blood glucose while exerting no effect at all on blood lactate levels.

MATERIALS AND METHODS

Reagents. TA-064, prenalterol hydrochloride, dobutamine hydrochloride, amrinone and terbutaline hemisulfate were synthesized at Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd. (Toda, Saitama, Japan). Isoproterenol hydrochloride was purchased from Nakarai Chemicals (Kyoto, Japan). Propranolol hydrochloride and practolol hydrochloride were purchased from I.C.I. Pharmaceuticals (Wilmslow, Cheshire, U.K.). Beta-

adrenergic compounds except TA-064 were in racemic form. Other reagents and their sources are: "Blood Sugar-GOD-Perid®-Test", lactate dehydrogenase, glycerokinase, pyruvate kinase, NAD, NADH and phosphoenolpyruvate sodium salt from Boehringer Mannheim GmbH (Mannheim, West Germany); Trasylol® from Bayer (Leverkusen, West Germany); "Insulin Eiken®" from Eiken Immunochemical Laboratory (Tokyo, Japan); "YAMASA Cyclic AMP Assay Kit®" and ATP disodium salt from Yamasa Shoyu Co., Ltd. (Choshi, Chiba, Japan); pancreatic glucagon-specific antibody 30K from the Department of Internal Medicine, the University of Texas Southern Medical School (Dallas, Texas, U.S.A.); ^{125}I-glucagon from Nuclear-Medical Lab. Inc. (Dallas, Texas, U.S.A.); fetal calf serum from Microbial Research Laboratory, Osaka University (Suita, Osaka, Japan); glucagon and streptozotocin from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.); and carboxymethylcellulose sodium salt (CMC) from Kokusan Kagaku (Tokyo, Japan).

Animals. Male Sprague–Dawley rats (7–10 weeks old, 180–270 g in body weight) were purchased from Shizuoka Laboratory Animal Agricultural Cooperation (Hamamatsu, Shizuoka, Japan). Diabetic rats were used 6 days after intravenous injection of 60 mg/kg of streptozotocin, when the average value of fasting blood glucose levels was 250 mg/100 ml.

All animals were fasted for 20 hr before use.

Preparation of drug solution or suspension. TA-064 for oral administration was suspended in 0.5% CMC solution. TA-064 and amrinone for intraperitoneal injection were dissolved in equimolar hydrochloric acid and diluted with saline to give desired concentrations. All other drugs were dissolved in saline. Vehicle (0.5% CMC or saline) was administered to control groups. All test compounds were administered in a volume of 5 ml/kg body weight.

Animal experiments. Test drugs were orally administered with a stomach tube or intraperitoneally injected to rats. Beta-adrenergic antagonists were subcutaneously injected 15 min before intraperitoneal administration of test drugs. Blood was collected from the tail tip of conscious rats for glucose determination or from the abdominal aorta under ether anesthesia for all the other determinations. For the lactate and glycerol determinations, blood was mixed with an equivolume of 10% perchloric acid and centrifuged at 1500 *g* for 30 min at 4°. The supernatant was neutralized with 5 M K_2CO_3 and centrifuged to remove the $KClO_4$ precipitate prior to the determinations.

Plasma for determination of insulin (IRI) and glucagon (IRG) was obtained by centrifugation of a mixture of 1 volume of blood and 1/10 volume of aprotinin (Trasylol®, 50,000 KIU/5 ml) containing ethylenediaminetetraacetic acid disodium salt (EDTA, 12 mg/ml). Diluted plasma for determination of cyclic AMP (c-AMP) was obtained by centrifugation of a mixture of 1 volume of blood and 10 volumes of 10 mM EDTA in saline.

Analyses. Glucose was enzymatically determined using the "Blood Sugar-GOD-Perid®-Test" kit. Lactate [13] and glycerol [14] were also enzymatically determined. FFA was determined by the method of Duncombe [15] modified by Itaya and Ui [16]. IRI, IRG, and c-AMP were radioimmunologically determined using the "Insulin Eiken®" kit, pancreatic glucagon-specific antibody 30K and "YAMASA Cyclic AMP Assay Kit®", respectively.

Statistical significance of difference was determined by Student's *t*-test.

RESULTS

Effects of oral administration of TA-064 on blood glucose and other metabolic parameters. Figure 1 shows time courses of changes in the levels of blood glucose, lactate, FFA, glycerol, plasma IRI, IRG and c-AMP after oral administration of TA-064 at a dose of 100 mg/kg to fasted rats. Although β-adrenergic agonists are known to elevate all these parameters when administered at relatively high doses [17], TA-064 unexpectedly caused a sustained hypoglycemia after a slight transient rise in blood glucose (Fig. 1A) and it did not affect blood lactate levels (Fig. 1B) while the rest of the parameters were all elevated in various time course patterns. The effects of TA-064 on FFA, glycerol, IRI and c-AMP levels were prompt giving peak values 15 min after administration followed by relatively steep decreases

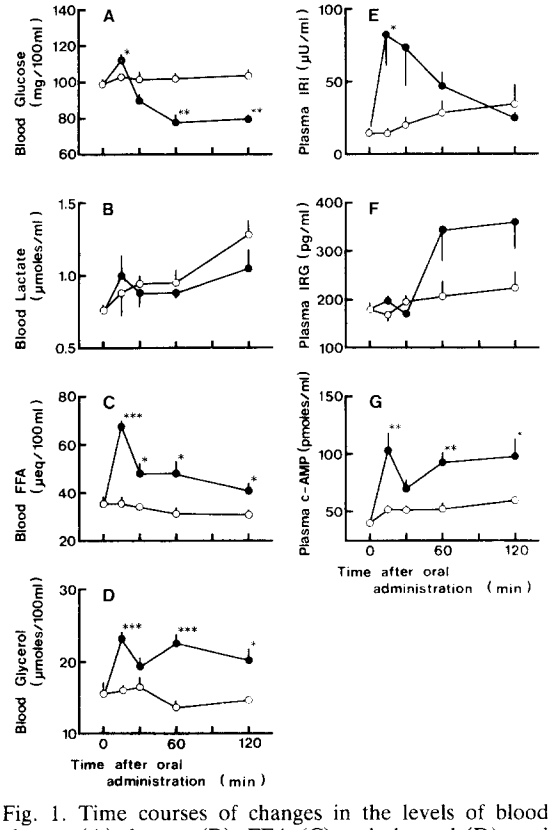

Fig. 1. Time courses of changes in the levels of blood glucose (A), lactate (B), FFA (C) and glycerol (D), and plasma IRI (E), IRG (F) and c-AMP (G) after oral administration of TA-064. TA-064 (100 mg/kg, ●) and vehicle (control, ○) were orally administered to male rats (7 w) fasted for 20 hr. Groups of 6 rats were sacrificed at each time point under ether anesthesia. Each point and bar represent the mean and S.E. Significant differences from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Figs. 1C, D, E and G), whereas the mean IRG level was markedly elevated only after 60 min although there were no statistically significant increases over the control values (Fig. 1F). The FFA, glycerol and c-AMP levels remained significantly elevated until 120 min after administration while IRI dropped to the control level by that time.

Relationships between the oral dose of TA-064 and the early changes in the levels of metabolic parameters 15 min after administration are shown in Fig. 2. As for blood glucose, a dose–response relationship for the hypoglycemic effect appearing 60 min after administration is also shown (Fig. 2A). Although the early transient rise of blood glucose was not observed at any dose level in this experiment, dose-dependent increases in blood FFA and glycerol and plasma IRI and c-AMP levels (Figs. 2B, C, D, and E) and a dose-dependency of the late hypoglycemic effect of TA-064 (Fig. 2A) were apparent.

Effects of intraperitoneal administration of TA-064 and known β-adrenergic and cardiotonic agents. Since the oral route is not satisfactory to study the time-course pattern of metabolic effects of a drug owing to variables in drug absorption and hepatic metabolism, effects of intraperitoneal injection of TA-064 on the circulating metabolites and hormones were investigated in comparison with other β-adrenergic and cardiotonic agents. Intraperitoneal injection of 10 mg/kg of TA-064 similarly produced persistent hypoglycemia following a slight insignificant rise in blood glucose (Fig. 3A). By contrast, comparable doses of another β_1-selective adrenergic cardiotonic, dobutamine, and a non-adrenergic cardiotonic, amrinone, both elevated blood glucose, although their hyperglycemic effects were relatively weak (Figs. 3B and C).

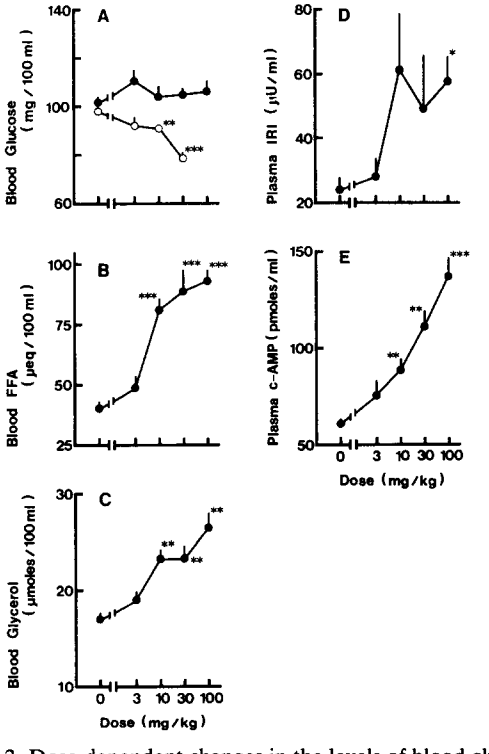

Fig. 2. Dose-dependent changes in the levels of blood glucose (A), FFA (B), glycerol (C), plasma IRI (D) and c-AMP (E) after oral administration of TA-064. TA-064 was orally administered to fasted male rats. Rats were sacrificed 15 (●) or 60 (○) min after the administration. Each point and bar represent the mean and S.E. of 4 to 6 animals. Significant differences from control (dose, 0): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

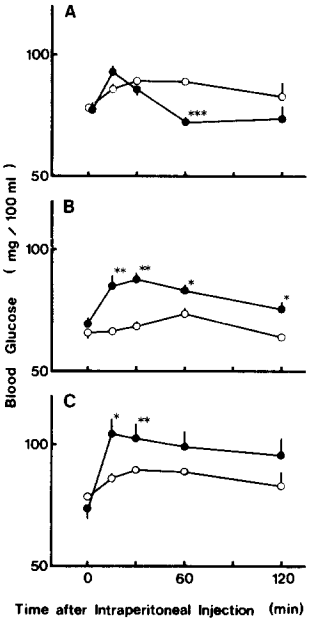

Fig. 3. Effects of TA-064 (10 mg/kg, A), dobutamine (10 mg/kg, B) and amrinone (30 mg/kg, C) on blood glucose levels. Drugs (●) were intraperitoneally injected to 4 to 5 fasted rats and blood was collected from the tail tip. Each point and bar represent the mean and S.E. Significant differences from control (○): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4 shows time courses of changes in the circulating levels of five metabolic parameters for 40 min after intraperitoneal injection of TA-064 and three other β-adrenergic agonists, isoproterenol, terbutaline and prenalterol. The doses were selected after preliminary dose–response studies so as to give characteristic metabolic effects of each drug and they were approximately proportionate to their cardiotonic doses [12]. As expected, the non-selective β-agonist isoproterenol and the selective β_2-agonist terbutaline markedly elevated blood glucose and lactate levels (Figs. 4A and B). The β_1-selective cardiotonic prenalterol slightly elevated both glucose and lactate levels at 20 min, whereas TA-064 depressed blood glucose significantly at 40 min without affecting blood lactate (Figs. 4A and B). Lipolysis was stimulated by isoproterenol, terbutaline and TA-064, but only insignificantly by prenalterol (Fig. 4C). Plasma IRI and IRG were both elevated by isoproterenol, terbutaline and TA-064, but the concentration peaks appeared earlier for IRG than for IRI (Figs. 4D and E). The plasma IRG levels suddenly dropped after the peaks in the isoproterenol and terbutaline groups as hyperglycemia developed. In the TA-064 group, the decline was slower and the level was still significantly higher than the control at 40 min, when the hypoglycemia became apparent

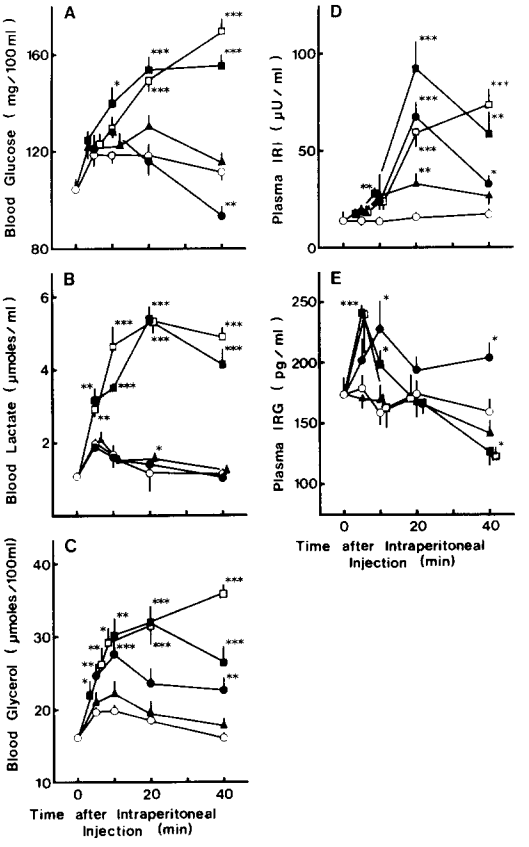

Fig. 4. Effects of β-adrenergic agonists on the levels of blood glucose (A), lactate (B) and glycerol (C) and plasma IRI (D) and IRG (E). TA-064 (10 mg/kg, ●), prenalterol (10 mg/kg, ▲), isoproterenol (0.1 mg/kg, ■) and terbutaline (1 mg/kg, □) were intraperitoneally injected to fasted rats. Groups of 6 to 7 rats were sacrificed at each time point. Each point and bar represent the mean and S.E. Significant differences from control (○): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(Fig. 4E). Likewise, the changes in blood glucose concentration were associated with the plasma IRI levels in the opposite direction (Fig. 4D). Prenalterol exerted the least stimulatory action on the pancreatic secretion, causing a significant increase in plasma IRI only at 20 min (Figs. 4D and E).

Effects of β-agonists on blood glucose and plasma IRG in streptozotocin-diabetic rats. When streptozotocin-diabetic rats devoid of functional pancreatic B cells were used, intraperitoneal injection of TA-064 did not lower blood glucose levels (Fig. 5), indicating that stimulation of insulin secretion was responsible for the hypoglycemia observed in intact rats. Isoproterenol markedly elevated the blood glucose level as in normal rats, while both TA-064 and prenalterol tended to give mean glucose levels higher than the control levels although none of the increases was statistically significant.

In a separate experiment, effects of intraperitoneal injection of TA-064 and isoproterenol on plasma IRG levels were examined in streptozotocin-diabetic rats. The plasma IRG levels 10 min after injection of TA-064 (10 mg/kg) and isoproterenol (0.1 mg/kg) were 206 ± 29 (N = 5) and 252 ± 39 (N = 3) pg/ml,

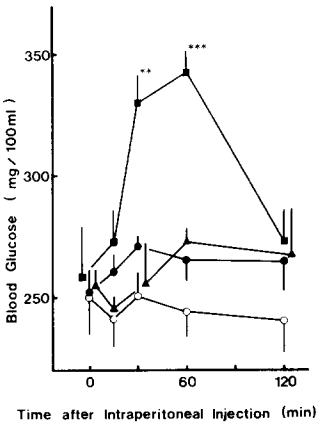

Fig. 5. Effects of β-adrenergic agonists on blood glucose levels in streptozotocin-diabetic rats. TA-064 (10 mg/kg, ●), prenalterol (10 mg/kg, ▲) and isoproterenol (0.1 mg/kg, ■) were intraperitoneally injected to 3 to 5 fasted diabetic rats. Each point and bar represent the mean and S.E. Significant differences from control (○): ** $P < 0.01$, *** $P < 0.001$.

respectively, the former being insignificantly higher and the latter significantly ($P < 0.05$) higher than the control (148 ± 21 pg/ml, N = 5).

Effects of β-adrenergic antagonists on the metabolic actions of isoproterenol and TA-064. The non-selective β-adrenergic antagonist propranolol is known to suppress all the metabolic effects of β-adrenergic agonists [1]. Propranolol pretreatment completely abolished the hyperglycemic effect of isoproterenol (Fig. 6B) as well as the early transient rise in blood

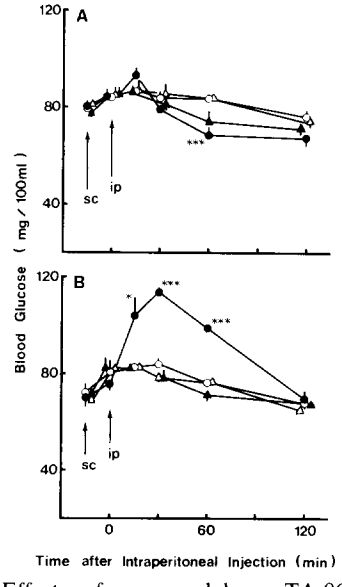

Fig. 6. Effects of propranolol on TA-064- and isoproterenol-induced changes in blood glucose levels in rats. Propranolol (triangles) at 10 (A) and 1 (B) mg/kg and saline (circles) were subcutaneously injected to 3 to 4 fasted rats 15 min before intraperitoneal injection of TA-064 at 10 mg/kg (A, filled symbols), isoproterenol at 0.1 mg/kg (B, filled symbols) or saline (open symbols). Each point and bar represent the mean and S.E. Significant effects of β-adrenergic agonist: * $P < 0.05$, *** $P < 0.001$.

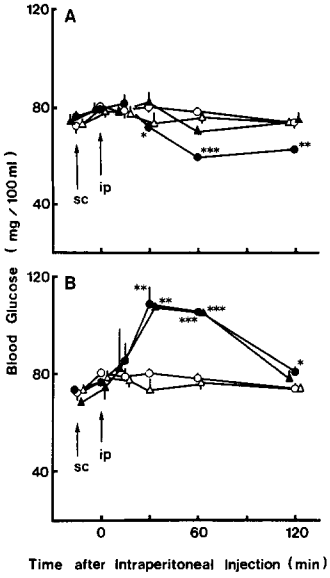

Fig. 7. Effects of practolol on TA-064- and isoproterenol-induced changes in blood glucose levels in rats. Practolol at 10 mg/kg (triangles) and saline (circles) were subcutaneously injected to 5 fasted rats 15 min before intraperitoneal injection of TA-064 at 10 mg/kg (A, closed symbols), isoproterenol at 0.1 mg/kg (B, closed symbols) or saline (open symbols). Each point and bar represent the mean and S.E. Significant effects of β-adrenergic agonist: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

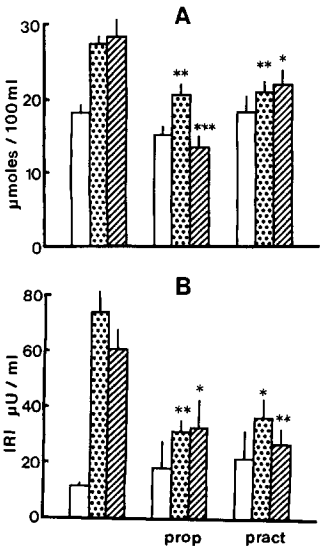

Fig. 8. Effects of β-adrenergic antagonists on TA-064- and isoproterenol-induced increases in blood glycerol (A) and plasma IRI (B) levels in rats. Propranolol (prop, 0.3 mg/kg), practolol (pract, 30 mg/kg) and saline were subcutaneously injected to 5 rats 15 min before intraperitoneal injection of TA-064 (10 mg/kg, dotted column) and isoproterenol (0.03 mg/kg, hatched column). Each bar represents S.E. of the mean. Significant effects of β-adrenergic antagonist: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

glucose in TA-064-treated rats (Fig. 6A). The late appearing hypoglycemic effect of TA-064 became less severe so that the glucose level was no longer significantly lower than the control level (Fig. 6A). The selective β_1-antagonist practolol, on the other hand, suppressed the hypoglycemic effect of TA-064 (Fig. 7A), but not the hyperglycemic action of isoproterenol (Fig. 7B).

Beta-adrenergic stimulation of lipolysis [3, 4] and insulin secretion [7, 8] are both considered to be mediated predominantly via β_1-receptor in rats. As expected, the elevations of blood glycerol and plasma IRI by TA-064 and isoproterenol were all suppressed by pretreatment with either propranolol or practolol (Fig. 8).

DISCUSSION

Beta-adrenergic stimulation is known to increase blood or plasma concentrations of glucose, lactate, glycerol, FFA [1], IRI, IRG [2], and c-AMP [18, 19]. Accumulated evidence indicates that β_2-stimulation of skeletal muscle is mainly responsible for the increases in blood glucose [20, 21] and lactate [5, 20, 21], and that the elevations of blood FFA and glycerol, the parameters of stimulated lipolysis, are brought about by stimulation of β-receptors on the adipocyte which are mainly, but not exclusively, of the β_1-type [3, 4]. There seem to be some species differences as to the subtype involved in β-adrenergic stimulation of pancreatic insulin secretion: β_1 in rat [7, 8] and β_2 in man [22], mouse [23] and dog [24, 25]. There was a suggestion that the exercise-induced glucagon secretion is mediated by β_2-receptors in rats [26].

In the present study we confirmed that the non-selective β-agonist isoproterenol, when injected intraperitoneally into fasted rats, increases the circulating concentrations of glucose, lactate, glycerol, IRI and IRG (Fig. 4), and that the increases in blood glycerol and plasma IRI concentrations by isoproterenol are suppressed by the selective β_1-antagonist practolol (Fig. 8), but its hyperglycemic effect is not (Fig. 7B).

Intraperitoneal injection of the selective β_2-agonist terbutaline at a dose ten times that of isoproterenol caused elevations of all the above metabolic parameters, whose time courses took similar patterns to those observed with isoproterenol (Fig. 4). Whether the stimulated lipolysis and insulin secretion were due to the coexistence of β_2-receptors on the rat adipocyte [27, 28] and the pancreatic B cell or due to incomplete β_2-selectivity on the drug side [29, 30] is not known.

TA-064, described as a β_1-adrenergic cardiotonic agent by Nagao *et al.* [12], exhibited some common metabolic effects as a β-agonist: it elevated dose-dependently the concentrations of blood FFA and glycerol and plasma IRI and c-AMP when administered orally or intraperitoneally to fasted rats (Figs. 1, 2 and 4), and the stimulated lipolysis and insulin secretion were both antagonized by pretreatment with propranolol and practolol (Fig. 8), thus qualifying these metabolic actions of TA-064 as β_1-adrenergic effects. The most striking differences of the pattern of metabolic effects of TA-064 from those of non-selective β-agonists such as isoproterenol and more numerously available β_2-agon-

ists are a sustained hypoglycemia and the lack of lactacidemia (Figs. 1A, B and 4A, B). Furthermore, the hypoglycemia induced by TA-064 was inhibited by pretreatment with propranolol and practolol (Figs. 6A and 7A). These features may not be surprising if one accepts that a β_2-component is responsible for hyperglycemia and lactacidemia after adrenergic stimulation and TA-064 possesses only a very weak or no β_2-adrenergic activity. The present result is the first demonstration that there can be a β_1-adrenergic agonist selective enough to cause hypoglycemia when administered to fasted animals. The increase in plasma IRI preceding the hypoglycemia in intact rats (Figs. 1E and 4D) and the disappearance of the hypoglycemia in streptozotocin-diabetic rats (Fig. 5) indicate that the hypoglycemia induced by TA-064 is only secondary to stimulation of insulin secretion. Adrenergic stimulation of insulin secretion has been reported to be mediated by the β_1-receptor on pancreatic B cells in rats [7, 8]. The hypoglycemic effect of TA-064 will be probably of little clinical significance, since the minimal oral hypoglycemic dose in rat (10 mg/kg) is much higher than the cardiotonic dose (10 mg/man) in man [31].

The early rise in plasma IRG after intraperitoneal injection of TA-064 in normal as well as in streptozotocin-diabetic rats (Fig. 4E and text) indicates that, like the other two β-agonists isoproterenol and terbutaline, TA-064 also stimulates pancreatic A cells, suggesting that β_1-receptors are also present on the pancreatic A cell. The late rises 60 and 120 min after oral and 40 min after intraperitoneal administration of TA-064 are likely to represent a secondary response to the hypoglycemia (Figs. 1F and 4E). Similarly, the sudden decreases in plasma IRG after peak stimulation by intraperitoneal injection of isoproterenol and terbutaline (Fig. 4E) are probably due to inhibition by the simultaneous hyperglycemia and insulinemia.

The selective β_1-adrenergic agonist prenalterol has been reported to cause moderate rises in plasma IRI and lipolytic products in man and no changes in blood glucose and lactate levels [10]. The present study showed that prenalterol also elevated plasma IRI significantly and blood glycerol slightly, although insignificantly, in rats (Figs. 4C and D). It should be stressed that these apparently β_1-adrenergic metabolic effects of prenalterol were weaker than those of TA-064, and that prenalterol tended to be slightly hyperglycemic and lactacidemic (Figs. 4A and B). Whether these effects are results of poorer separation of β_1 from β_2 compared with TA-064 or due to its metabolic activities unrelated to the adrenergic system is not resolved at present. Also unknown are mechanisms of the hyperglycemic effects of the β_1-selective cardiotonic dobutamine and the non-adrenergic cardiotonic amrinone (Figs. 3B and C). Dobutamine has been reported to bind not only to β_1- but also to β_2- and α-adrenergic receptors [32] and to elicit α- and β_2-effects [33]. Amrinone is known to inhibit c-AMP phosphodiesterase in toad urinary bladder [34] and elevate c-AMP levels in muscle *in vitro* [35, 36]. Hyperglycemic effects of α-adrenergic agents [1] and c-AMP phosphodiesterase inhibitors [37, 38] have been reported.

Although the early transient rise in blood glucose was slight and statistically significant only in one experiment (Fig. 1A), all TA-064 administration experiments showed this effect except when the animals were pretreated with propranolol and practolol (Figs. 6A and 7A). This may mean that the transient hyperglycemia is also a β_1-receptor-mediated phenomenon. One possibility is that the early temporary rise in plasma IRG (Fig. 4E) may have augmented hepatic gluconeogenesis [39, 40] in fasted rats and the elevated glucose production may have resulted in a transient increase in blood glucose before the hypoglycemic action of gradually increasing levels of plasma insulin overcome the weakly hyperglycemic component of β_1-adrenergic stimulation in fasted animals depleted of liver glycogen (Figs. 4D and E).

The sustaining hyperglycemia in the presence of hyperinsulinemia after treatment with β_2-agonists would, then, have to be explained by reduced glucose uptake [41, 42] or increased lactate (a material source for hepatic gluconeogenesis) production [41] or both in peripheral tissues, especially in skeletal muscles whose adrenergic receptors are predominantly of the β_2-type [21].

In summary, the hypoglycemic effect of TA-064 is unique among known β-adrenergic agonists which are largely hyperglycemic and lactacidemic because of their β_2-component. This hypoglycemia is probably a response secondary to the stimulation of pancreatic insulin secretion mediated by β_1-adrenergic stimulation in fasted rats. Whether the hypoglycemic activity is a criterion of β_1-selectivity will have to be determined in experiments using β_2-selective antagonists.

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REFERENCES

1. J. Himms-Hagen, *Pharmac. Rev.* **19**, 367 (1967).
2. S. C. Woods and D. Porte, Jr., *Physiol. Rev.* **54**, 596 (1974).
3. A. M. Lands, A. Arnold, J. P. McAuliff, E. P. Luduena and T. G. Brown, Jr., *Nature, Lond.* **214**, 597 (1967).
4. A. M. Lands, F. P. Luduena and H. J. Buzzo, *Life Sci.* **6**, 2241 (1967).
5. A. Arnold and W. H. Selberis, *Experientia* **24**, 1010 (1968).
6. A. T. Høstmark and R. S. Horn, *Biochem. Pharmac.* **24**, 985 (1975).
7. B. L. Furman and F. M. Tayo, *J. Pharm. Pharmac.* **26**, 512 (1974).
8. Y. Saito, Y. Irie, T. Hosokawa, T. Igawa, F. Hashimura and H. Kohri, *Biochem. Pharmac.* **27**, 2531 (1978).
9. E. Carlson, C.-G. Dahlöf, A. Hedberg, H. Persson and B. Tångstrand, *Naunyn-Schmiedeberg's Archs Pharmac.* **300**, 101 (1977).
10. O. Ronn, E. Fellenius, C. Graffner, G. Johnsson, P. Lundborg and L. Svensson, *Eur. J. clin. Pharmac.* **17**, 81 (1980).

11. R. H. Lockwood and B. K. Lum, *Life Sci.* **14**, 73 (1974).
12. T. Nagao, T. Ikeo, M. Sato, H. Nakajima and A. Kiyomoto, *Eighth int. Congr. Pharmac.* p. 499 (1981).
13. I. Gutmann and A. W. Wahlefeld, in *Methods of Enzy-Enzymatic Analysis,* 2nd English edn. (ed. H. U. Bergmeyer) Vol. 4, p. 1464. Academic Press, New York (1974).
14. M. Eggstein and E. Kuhlmann, in *Methods of Enzymatic Analysis,* 2nd English edn. (ed. H. U. Bergmeyer), Vol. 4, p. 1425. Academic Press, New York (1974).
15. W. G. Duncombe, *Biochem. J.* **83**, 6P (1962).
16. K. Itaya and M. Ui, *J. Lipid Res.* **6**, 16 (1965).
17. D. C. Kvam, D. A. Riggilo and P. M. Lish, *J. Pharmac. exp. Ther.* **149**, 183 (1965).
18. A. E. Broadus, N. I. Kaminsky, R. C. Northcutt, J. G. Hardman, E. W. Sutherland and G. W. Liddle, *J. clin. Invest.* **49**, 2237 (1970).
19. R. C. Strange and O. D. Mjøs, *Eur. J. clin. Invest.* **5**, 147 (1975).
20. A. Arnold, J. P. McAuliff, D. F. Colella, W. V. O'Connor and T. G. Brown, Jr., *Archs int. Pharmacodyn.* **176**, 451 (1968).
21. A. Arnold, *Il Farmaco. ed. Sci.* **27**, 79 (1972).
22. T. Williams-Olsson, E. Fellenius, P. Björntorp and U. Smith, *Acta med. scand.* **205**, 201 (1979).
23. B. Ahrén and I. Lundquist, *Eur. J. Pharmac.* **71**, 93 (1981).
24. A. Loubatières, M. M. Mariani, G. Sorel and L. Savi, *Diabetologia* **7**, 127 (1971).
25. A. Kaneto, E. Miki and K. Kosaka, *Endocrinology* **97**, 1166 (1975).
26. A. S. Luyckx and P. J. Lefebvre, *Diabetes* **23**, 81 (1974).
27. M. J. Daly, *Trends pharmac. Sci.* **2**, 168 (1981).
28. D. G. Haylett, in *Trends in Autonomic Pharmacology* (ed. S. Kalser), Vol. 1, p. 374. Urban and Schwarzenberg, Baltimore (1979).
29. K. P. Minnemann, L. R. Hegstrand and P. B. Molinoff, *Molec. Pharmac.* **16**, 21 (1979).
30. G. Leclerc, B. Rouot, J. Velly and J. Schwartz, *Trends pharmac. Sci.* **2**, 18 (1981).
31. M. Kino, Y. Hirota, S. Yamamoto, K. Sawada, M. Moriguchi, M. Kotaka, S. Kubo and K. Kawamura, *Am. J. Cardiol.* **51**, 802 (1983).
32. R. S. Williams and T. Bishop, *J. clin. Invest.* **67**, 1703 (1981).
33. E. H. Sonnenblick, W. H. Frishman, and T. H. LeJemtel, *New Engl. J. Med.* **300**, 17 (1979).
34. S. D. Levine, M. Jacoby, J. A. Satriano and D. Schlondorff, *J. Pharmac. exp. Ther.* **216**, 220 (1981).
35. K. D. Meisheri, R. F. Palmer and C. Van Breemen, *Eur. J. Pharmac.* **61**, 159 (1980).
36. P. Honerjäger, M. Schäfer-Korting and M. Reiter, *Naunyn-Schmiedeberg's Archs Pharmac.* **318**, 112 (1981).
37. S. Hynie, G. Krishna and B. B. Brodie, *J. Pharmac. exp. Ther.* **153**, 90 (1966).
38. L. Triner and G. G. Nashas, *J. Pharmac. exp. Ther.* **153**, 569 (1966).
39. N. Kalant, *Proc. Soc. exp. Biol. Med.* **86**, 617 (1954).
40. J. H. Exton and C. R. Park, *Pharmac. Rev.* **18**, 181 (1966).
41. O. Walaas and E. Walaas, *J. biol. Chem.* **187**, 769 (1950).
42. I. G. Sloan, P. C. Sawh and I. Bihler, *Molec. cell. Endocr.* **10**, 3 (1978).