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EFFECT OF 2-(4-BENZYL-PIPERIDINO)-1-(4-HYDROXYPHENYL)-1-PROPANOL ON BLOOD GLUCOSE CONCENTRATION

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Abstract—In finding that 2-(4-benzyl-piperidino)-1-(4-hydroxyphenyl)-1-propanol (BPHPP) produced high concentrations of blood glucose in rats, it was observed that (1) the maximum concentration of blood glucose was obtained 1 hr after oral administration at a dose of 100 mg/kg of BPHPP, (2) that BPHPP has no effect on the secretion of adrenal corticosterone, (3) that BPHPP markedly inhibited cAMP phosphodiesterase activity, *in vitro*, and (4) that in the preliminary experiment, the hepatic cAMP levels increased 1.7-fold by the administration (100 mg/kg) of BPHPP.

From these results, it was presumed that the high concentrations of blood glucose may be the results of elevations in the intracellular cAMP levels due to the inhibitory action of BPHPP on cAMP phosphodiesterase.

Carron *et al.* [1] reported the synthesis and pharmacological characteristics of 2-piperidinoalkanol derivatives, and found 2-(4-benzyl-piperidino)-1-(4-hydroxyphenyl)-1-propanol (BPHPP, Fig. 1) as the most potent vasodilator. BPHPP exhibits a protective effect against cyanide toxicity determined by lethality, decline in respiration, blood pressure, and electroencephalogram in rats, guinea pigs, rabbits, and mice [2]. Recently, BPHPP was proposed to be effective in treatment for human cerebrovascular disease by double blind trials [3]. The mechanisms of the pharmacological actions, however, remain unknown.

In studying the biological effects of this agent, we found that BPHPP brought about high concentrations of blood glucose in rats.

In this paper, these changes in blood glucose concentration following BPHPP administration into rats are described. Furthermore, it will be seen that BPHPP inhibits cyclic adenosine 3′,5′-monophosphate (cAMP) phosphodiesterase prepared from rat liver, and that it elevates the hepatic cAMP levels.

MATERIALS AND METHODS

Male rats of the Wistar strain, weighing from 180 to 200 g, were maintained on an Oriental Yeast solid diet *ad lib.*, and were allowed free access to drinking water. The animals were fasted overnight prior to decapitation. BPHPP was prepared by the method of Carron *et al.* [1] and used as its L-tartaric acid salt. [^{3}H]cAMP was obtained from New England Nuclear Corp. Snake venom was obtained from Sigma Chemical Co. All the other reagents were purchased from Nakarai Chemicals, Kyoto.

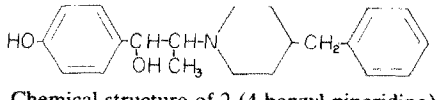

Fig. 1. Chemical structure of 2-(4-benzyl-piperidino)-1-(4-hydroxyphenyl)-1-propanol.

Blood glucose assay. Blood glucose was measured by the glucose oxidase method [4].

Serum corticosterone assay. Serum corticosterone was assayed by acid fluorescene [5].

Assay of hepatic cAMP levels. The rats were killed by decapitation. The liver (3 g) was immediately removed, placed in 10 ml of ice-cold 10% trichloroacetic acid, and then homogenized at full speed for 2 min in a Teflon homogenizer. The homogenate was then centrifuged at 8500 *g* for 30 min. The supernatants were extracted 3 times with 8 vol. of ether. The extracts were then dried and redissolved in 0.02 M phosphate buffer, pH 7.5. The cAMP assay was done by using Gilman's protein binding method [6].

Preparation and assay of cAMP phosphodiesterase. The liver was isolated immediately after decapitation, homogenized in 5 vol of chilled 0.25 M sucrose solution, and centrifuged at 105,000 *g* for 120 min. An aliquot of the supernatant solution, obtained by centrifugation at 105,000 *g* for 120 min, was applied to an Agarose A-5m column (2.5 × 90 cm) which was equilibrated with a 0.05 M Tris–Cl buffer, pH 7.5, containing 5 mM $MgCl_2$. The elution was carried out with same buffer. The flow rates for the gel filtration did not exceed 10 ml for 30 min. When 10^{-4} M cAMP was employed as substrate, the single fractions displaying phosphodiesterase activity were isolated in the manner described by Thompson and Appleman [7]. The assay for phosphodiesterase activity was carried out by the two stage isotopic procedure [7]. The first stage reaction mixture of 0.5 ml contained 50 μmoles Tris–Cl buffer, pH 7.5, 2.5 μmoles $MgCl_2$, 2.5 mg of bovine serum albumin, 50 nmoles [^{3}H]cAMP (120,000 c.p.m.), and an appropriate concentration of the enzyme. The reaction was initiated by the addition of [^{3}H]cAMP. After incubation for 10 min at 37°, the tubes con-

taining the reaction mixture were transferred to a boiling water bath for 2 min to terminate the enzyme activity. The reaction mixture was then further incubated with sufficient snake venom for 30 min at 37°. The reaction was stopped by the addition of 0.8 ml of a 1:1 slurry Bio-Red resin AG 1 × 2, 200–400 mesh. The amount of radioactivity of [^{3}H]adenosine left in the supernatant after centrifugation in a clinical centrifuge was measured by means of a liquid scintillation spectrometer. All assays were carried out at 20% or less total reaction to be in linear portion to the enzyme assay. One unit of enzyme activity was defined as that cleaving one nmole of cAMP in one hr. Protein was determined by the method of Lowry *et al.* [8].

Statistical analysis. Student's *t*-test was used for statistical evaluation of the results.

RESULTS

Effect of BPHPP on blood glucose concentration

The increases in blood glucose observed at 1 hr following BPHPP ingestion are shown in Table 1. Although such small ingestion of BPHPP as 5 mg per kg caused only slight but significant increase in glucose level, large ingestions of this agent brought about levels as high as 120–180 per cent, when compared with the controls. To discover the time response, the estimations of the blood glucose levels following the administration of BPHPP was carried out at the times indicated. As illustrated in Fig. 2, the maximum glucose level was observed 1 hr after the ingestion of BPHPP. Furthermore, we confirmed that L-tartrate had no effect on glucose level.

Effect of BPHPP on serum corticosterone levels

To confirm the fact that BPHPP does not promote the secretion of glucocorticoids following ingestion, the changes in serum corticosterone were estimated. The results in Table 2 indicate that BPHPP has no effect on the secretion of adrenal corticosterone.

Effect of BPHPP on cAMP phosphodiesterase activity. Regarding the effect of BPHPP on cAMP metabolism as important, we carried out the following investigation. As shown in Fig. 3, BPHPP predominantly inhibits partially purified enzyme activity

Table 1. Effect of BPHPP on blood glucose concentration

Ingested (mg/kg)	Glucose (mg/dl)
Control	105 ± 4.3
5	112 ± 2.8
25	120 ± 2.5*
50	150 ± 6.4**
100	180 ± 5.8**

BPHPP was ingested into stomach at different doses as indicated. All animals were sacrificed by decapitation 1 hr after administration. Serum was obtained by centrifugation in a clinical centrifuge, and used for glucose assay. Each glucose value shows the mean of five animals ± S.E. A single or double asterisk indicates significant difference from control at $P < 0.05$ or $P < 0.01$, respectively.

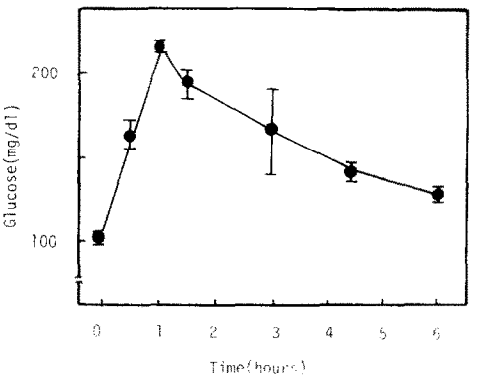

Fig. 2. Time course of blood glucose concentrations following BPHPP ingestion. All animals received 100 mg/kg of BPHPP before sacrifice. Vertical lines designate standard errors. Each point represents the mean value of five animals.

just as theophylline does. The concentrations of BPHPP needed for half maximum inhibition were seen to be about 2 mM. This value is almost three times of the value obtained by theophylline. To make certain that the inhibition was not due to the influence of these agents on the conversion of 5′-AMP to adenosine by the snake venom added to the 2nd stage reaction mixture, BPHPP was added to the 2nd stage incubation mixture at a concentration of 5 mM, however, no detectable effect of this agent could be observed. To see the effect of BPHPP on the K_m value for the substrate, Lineweaver–Burk plots, in the presence and absence of BPHPP at a constant level, were made with the partially purified rat liver phosphodiesterase (Fig. 4). The nature of the inhibition by BPHPP seems simple, as the Lineweaver–Burk plots give straight lines and reaction isnon-competitive, with a K_m value of 0.67×10^{-4} M. This value is identical with that reported by Thompson and Appleman [7].

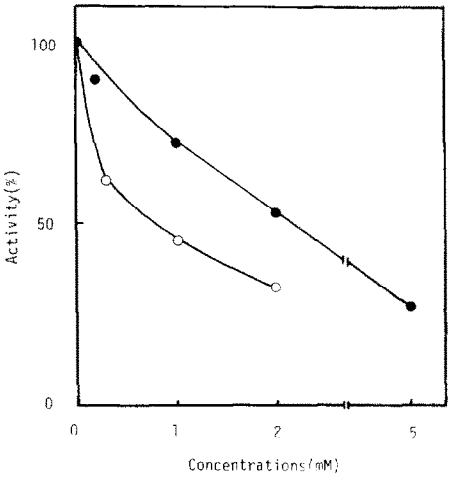

Fig. 3. Effect of BPHPP and theophylline at various concentrations on cAMP phosphodiesterase activity. Enzyme assay was carried out using the standard assay procedure with 29.9 units of partially purified liver enzyme preparation. ●——● BPHPP, and ○——○ theophylline.

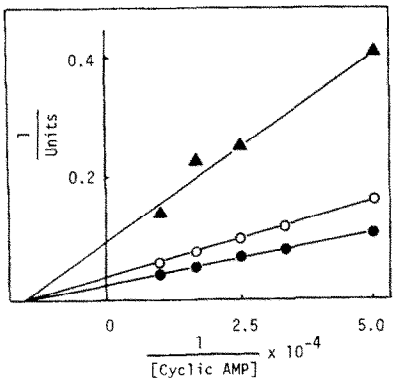

Fig. 4. Double reciprocal plots of the initial velocity of cAMP phosphodiesterase vs cAMP concentration at a constant BPHPP level. The initial reaction velocity was determined by utilizing a two stage isotopic procedure with 24.4 units of partially purified enzyme preparation. BPHPP concentrations were: ●—● none, ○—○ 2 mM, and ▲—▲ 5 mM.

Increase in cAMP levels following BPHPP administration

It is of special interest to see whether BPHPP produces a significant effect on the intracellular cAMP levels *in vivo* or not. A preliminary hepatic cAMP assay following the oral administration of BPHPP was carried out. As seen in Fig. 5, the hepatic cAMP levels increased 1.7-fold by the administration of this agent.

DISCUSSION

The elevation in blood glucose concentration following the ingestion of BPHPP is an interesting phenomenon. Generally, such several compounds as cAMP, epinephrine, insulin, glucagon, and glucocorticoid play a control role in the regulation of blood glucose concentration. Furthermore, the effects of glucagon and probably epinephrine, on gluconeogenesis are mediated by intracellular cAMP accumulation through the stimulation of adenylate cyclase [9]. Especially, gluconeogenesis by cAMP is further enhanced in the presence of glucocorticoid [10]. Therefore, a knowledge of the changes in the intracellular cAMP levels occuring after the administration of BPHPP is important in seeking to resolve the cause of the high glucose levels brought about by this agent.

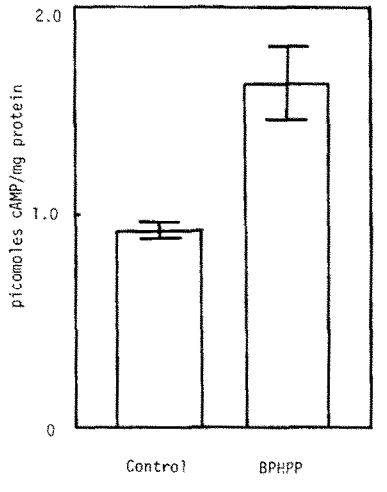

Fig. 5. Increase in hepatic cAMP levels following BPHPP ingestion. BPHPP (100 mg/kg) was ingested into stomach 30 min before sacrifice. cAMP assay was done as described under Materials and Methods. Each column and vertical line represents the mean value ± S.E. of five experiments. BPHPP group indicates significant difference in cAMP level from control at $P < 0.01$.

Table 2. Effect of BPHPP on blood glucose and corticosterone levels

Treatment	Glucose (mg/dl)	Corticosterone (μg/dl)
Control	117.4 ± 4.7	28.5 ± 1.1
BPHPP	207.3 ± 9.2**	29.3 ± 1.3

BPHPP (100 mg/kg) was ingested into rat stomach 45 min before sacrifice. Serum was obtained as described in the legend to Table 1, and used for glucose and corticosterone assay. Each value shows the mean of five animals ± S.E. A double asterisk indicates significant difference from control at $P < 0.01$.

BPHPP does not stimulate the secretion of the corticosterone, but produces hepatic intracellular cAMP levels (Table 2, Fig. 5). Evidence is now accumulating to the effect that cAMP mediates the positive inotropic action of such agent as theophylline, caffeine, and L-bromo-LSD, which have been shown to inhibit phosphodiesterase in mammals [11]. It has also been observed that BPHPP increases coronary blood flow [12,13] as theophylline does [14]. Although the significance *in vivo* of cAMP phosphodiesterase inhibition by BPHPP *in vitro* is unclear, one can speculate that BPHPP would participate in the regulation of the intracellular cAMP levels through its inhibitory action on cAMP phosphodiesterase. As reported here, the increasing rises in the cAMP levels caused by BPHPP are not so remarkable; however, it is known that an elevation as slight as 36 per cent of the steady state levels of intracellular cAMP induced by papaverine, one of the potent inhibitors of cAMP phosphodiesterase, produces several remarkable biological and pharmacological actions [11].

To further clarify the effect of this agent on glucose metabolism, we are now attempting to discover the changes it brings about in the epinephrine, insulin and glucagon levels in rats.

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