

## Notes

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**Studies on 3,7-Dimethyl-1-(5-oxo-hexyl)-xanthine (BL 191). III.  
Effects of BL 191 and Its Metabolites on Cyclic 3',5'-  
Nucleotide Phosphodiesterase and Lipolytic Activities<sup>1)</sup>**

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3,7-Dimethyl-1-(5-oxo-hexyl)-xanthine (BL 191) and its major urinary metabolites were examined for the inhibitory effect on the cyclic 3',5'-nucleotide phosphodiesterase (PDE) activity and for the stimulating effect on lipolysis in rat epididymal fat pads *in vitro*. In addition, the relationship between these effects of BL 191 and plasma level of free fatty acid (FFA) was investigated in rats.

The addition of BL 191 at 2 mM to the incubation mixture inhibited the activity of PDE about 50% and evoked a lipolytic activity 1.5-fold, and BL 191 and theophylline exerted approximately the same effects on PDE and lipolysis. Furthermore, the PDE inhibition and lipolytic response evoked by metabolites I and II possessing an oxohexyl group at the side chain were slightly less than those by BL 191, and metabolites III and IV having a carboxyl group at the side chain had no effects on PDE activity and lipolysis.

When BL 191 was given orally, plasma FFA level was elevated in the blood taken 30 min after the administration, increased linearly until 1 hr after the administration when the level was nearly 1.6 times higher than the basal level, and remained at this level till 2 hr after the administration.

The above-described results suggested that BL 191 along with its metabolites I and II by the oral administration of BL 191 inhibited the PDE activity and secondarily stimulated the lipolytic activity within the living body.

It was reported that xanthine derivatives such as caffeine and theophylline stimulate the lipolytic response in epididymal fat pads.<sup>3)</sup> Sutherland, *et al.*<sup>4)</sup> proposed 4 criteria to prove cyclic 3',5'-adenosine monophosphate (c-AMP) to be a mediator in certain kinds of hormonal responses like the stimulation of lipolytic activity, and demonstrated that hormonal effects might be enhanced by methylxanthines.

In our earlier paper<sup>5)</sup> of this series on 3,7-dimethyl-1-(5-oxo-hexyl)-xanthine (BL 191), a xanthine derivative, we have reported on the inhibitory effect of BL 191 upon the activity of cyclic 3',5'-nucleotide phosphodiesterase (PDE) in the 105000 × *g* supernate prepared from rat cerebral cortex and heart muscle in comparison with that of theophylline and that of caffeine. In addition, we reported that BL 191 stimulated the lipolytic response in fat pads and that simultaneously c-AMP levels in the same tissues increased.<sup>1)</sup>

The present paper is concerned with the relationship between the stimulation of lipolysis by BL 191 and the activity of PDE in the 105000 × *g* supernate from rat fat pads and with the effects of major metabolites in rat urine upon the lipolysis and PDE activity.

1) Part II: S. Hayashi and T. Sakaguchi, *Chem. Pharm. Bull.* (Tokyo), **23**, 3119 (1975).

2) Location: a) 1-3-2 Minamidai, Kawagoe-shi, 350, Japan; b) Aobayama, Sendai-shi, 980, Japan.

3) S. Hynie, G. Krishna, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **153**, 90 (1966); B. Weiss, J.I. Davies, and B.B. Brodie, *Biochem. Pharmacol.*, **15**, 1553 (1966).

4) E.W. Sutherland and G.A. Robison, *Pharmacol. Rev.*, **18**, 145 (1966); E.W. Sutherland, G.A. Robison, and R.W. Butcher, *Circulation*, **37**, 279 (1968).

5) S. Hayashi and H. Ozawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 587 (1974).

## Experimental

**Animal**—Male Sprague-Dawley rats (CLEA Japan, Inc.) weighing 180–250 g were used in all experiments.

**Enzyme Preparation for the Determination of PDE Activity**—The animals were decapitated, and immediately the epididymal fat pads were removed and homogenized in 4 volumes of icecold 0.32 M sucrose containing 50 mM Tris-HCl buffer (pH 7.4) by use of a teflon homogenizer. The homogenate was then subjected to a conventional procedure and  $105000 \times g$  supernate was used as an enzyme preparation.

**Determination of PDE Activity**—PDE activity was determined by use of  $360 \mu\text{M } ^{14}\text{C-c-AMP}$  (0.2  $\mu\text{Ci}$ ) as substrate according to a modification<sup>5)</sup> of the method of Pösch, *et al.*,<sup>6)</sup> unless otherwise described. Incubation mixture contained 300–400  $\mu\text{g}$  of protein, and the incubation was carried out at 37° for 40 min. PDE activity was expressed as  $n$  moles c-AMP hydrolyzed per mg protein per hr.

**Protein Determination**—Protein contents were determined by the method of Lowry, *et al.*<sup>7)</sup> by the use of serum albumin as standard.

**Determination of Lipolysis in Epididymal Fat Pads**—Epididymal fat pads were removed from the rats immediately after the decapitation. The pads weighing about 100 mg were incubated for 2 hr at 37° in 2 ml of Krebs-Ringer bicarbonate buffer containing 4% bovine serum albumin (fraction V, Sigma Co.) which was equilibrated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Free fatty acids (FFA) released into the incubation mixture were determined by the method of Itaya and Ui.<sup>8)</sup> Lipolytic activity was expressed as  $\mu$  moles FFA per g tissue per hr.

**Determination of Plasma FFA Level**—The rats were deprived of food for 24 hr before the oral administration of BL 191 (400 mg/kg). After decapitation, blood was collected into a heparinized syringe and centrifuged at 2500 rpm for 10 min to obtain plasma. The level of FFA in the plasma was determined by the method of Itaya and Ui.<sup>8)</sup>

## Results and Discussion

### Effects of BL 191 and Its Urinary Metabolites on PDE and Lipolysis in Rat Fat Pads

Fujimoto, *et al.*<sup>9)</sup> reported that BL 191 was maximally detected in the blood from 10 min to 1 hr after the administration of a single oral dose (400 mg/kg) of BL 191, and that BL 191 was rapidly absorbed to a considerable extent from the stomach as well as from the duodenum and the small intestine of the rat with the ligated pylorus. Furthermore, it was reported by these authors that the metabolites (Table I) were detected in the urine taken 24 hr after the oral administration of BL 191, largely as metabolites II and IV, and quite slightly as metabolites I and III, the amount of unchanged BL 191 being approximately 4% of the total excretion.<sup>9)</sup>

In this study, the effects of BL 191 and its urinary metabolites upon PDE activity (inhibition) and lipolysis (stimulation) in rat epididymal fat pads were examined in comparison with those of theophylline, an already-known PDE inhibitor. The addition of BL 191 at 2 mM to the incubation mixture inhibited the activity of PDE by 49% and evoked a lipolytic activity 1.5-fold. It was confirmed that BL 191 and theophylline exerted approximately the same effects on PDE and lipolysis. Furthermore, the PDE inhibition and lipolytic response evoked by metabolites I and II possessing an oxohexyl group at the side chain were slightly less than those by BL 191, and metabolites III and IV having a carboxyl group at the side chain had no effects on PDE activity and lipolysis.

It has been suggested that lipolysis may be increased by stimulating the activity of adenyl cyclase or by depressing that of PDE, and that the breakdown of triglycerides to FFA in fat pads may be mediated by c-AMP in the same tissues.<sup>3,10)</sup> In our previous paper,<sup>1)</sup> it has been shown that the incubation of rat epididymal fat pads with BL 191 in Krebs-Ringer bicarbonate

6) G. Pösch, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **268**, 299 (1971).

7) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

8) K. Itaya and M. Ui, *J. Lipid Res.*, **6**, 16 (1965).

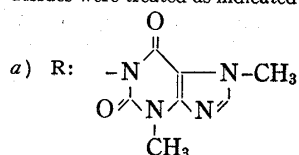
9) K. Fujimoto, S. Yoshida, Y. Moriyama, and T. Sakaguchi, *Chem. Pharm. Bull. (Tokyo)*, **24**, 1137 (1976).

10) S. Hynie, G. Krishna, and B.B. Brodie, *Federation Proc.*, **24**, 188 (1965); B.B. Brodie, J.I. Davies, S. Hynie, G. Krishna, and B. Weiss, *Pharmacol. Rev.*, **18**, 273 (1966).

TABLE I. Effects of BL 191 and Its Metabolites on PDE Activity and Lipolysis in Rat Epididymal Fat Pads

Drugs ( $2 \times 10^{-3}$ M)	PDE activity ( $n$ moles c-AMP hydrolyzed/mg protein/hr)	Inhibition of PDE (%)	FFA output ( $\mu$ moles/g tissue/hr)
None	186	—	$2.2 \pm 0.4^b$
Theophylline	85	54	$3.2 \pm 0.2$
BL 191 $\text{CH}_3\text{-C}(\text{O})\text{-(CH}_2)_4\text{-R}^a$	95	49	$3.3 \pm 0.3$
Met. I $\text{CH}_3\text{-CH}(\text{OH})\text{-(CH}_2)_4\text{-R}$	123	34	$2.8 \pm 0.2$
Met. II $\text{HO-CH}_2\text{-CH}(\text{OH})\text{-(CH}_2)_4\text{-R}$	110	41	$2.6 \pm 0.3$
Met. III $\text{HOOC-(CH}_2)_4\text{-R}$	192	0	$2.3 \pm 0.3$
Met. IV $\text{HOOC-(CH}_2)_3\text{-R}$	176	5	$2.0 \pm 0.2$

Tissues were treated as indicated in the text for determination of PDE activity and lipolysis.



b) Numbers show mean  $\pm$  S.E. ( $n=4-6$ ).

buffer increased the tissue level of c-AMP several-fold as compared with the level in the absence of BL 191.

Therefore, it is reasonable to presume that the stimulating effects of BL 191 and metabolites I and II on lipolytic response were mediated by the increased level of c-AMP due to the depression of PDE activity by BL 191 and the metabolites.

### Effects of BL 191 on Plasma FFA Level

The time-course of plasma FFA levels in the rat to which 400 mg/kg of BL 191 was orally administered after 24-hr fasting is shown in Fig. 1. When BL 191 was given by oral route, plasma FFA level taken 30 min after the administration was elevated, increased linearly until 1 hr after the administration when the level was nearly 1.6 times higher than the basal level, and remained at this level till 2 hr after the administration.

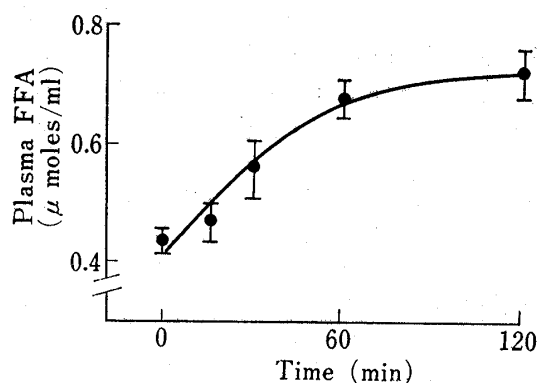


Fig. 1. Time Course of Plasma FFA Level after Administration of BL 191

Rats were deprived of food for 24 hr before the oral administration of BL 191 (400 mg/kg). Each point represents the mean  $\pm$  S.E. of 5-6 rats.

These results, together with the fact reported by Fujimoto, *et al.*<sup>9)</sup> that BL 191 was partly absorbed from the stomach as well, appeared in the blood quite soon after the administration and showed its maximal level in the blood taken 1 hr after the administration, indicate that plasma BL 191 level is almost parallel to FFA level in plasma. Therefore, it may be inferred that BL 191 along with its metabolites I and II increased the tissue level

of c-AMP by inhibiting the activity of PDE in epididymal fat pads, secondarily enhanced the breakdown of triglycerides to FFA, and consequently increased the level of FFA released in the blood.