increased the levels of the both cyclic nucleotides, however, dibutyryl cyclic AMP inhibited the contraction produced by dibutyryl cyclic GMP, dose dependently. These results suggest that cyclic AMP increased by an inhibition of cyclic AMP phosphodiesterase inhibits the stimulating action of cyclic GMP produced by an inhibition of cyclic GMP phosphodiesterase. We thus conclude that the nonspecific inhibitory action of papaverine on the guinea pig taenia is mediated by an increase in cyclic AMP, as a result of inhibition of cyclic AMP phosphodiesterase. On the other hand, the nonspecific antispasmodic action of Aspaminol is not related with the intracellular levels of cyclic AMP and cyclic GMP.

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STUDIES ON 3,7-DIMETHYL-1-(5-OXO-HEXYL)-XANTHINE (BL 191): THE INHIBITORY EFFECT OF BL 191 ON PDE IN VARIOUS TISSUES OF RATS

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Since Sutherland and Rall (1) reported that adenosine 3',5'-monophosphate (cyclic AMP) was hydrolysed to 5'-AMP by tissue extracts from several sources, many investigations have been made as regards the inhibitory effects of xanthine derivatives like caffeine and

theophylline on cyclic 3',5'-nucleotide phosphodiesterase (PDE), an enzyme which catalyzes the breakdown of cyclic AMP to 5'-AMP (2-6).

In our paper of this series on 3,7-dimethyl-1-(5-oxo-hexyl)-xanthine (BL 191), it was shown that BL 191 decreased the activity of PDE from rat cerebral cortex and heart muscle as did caffeine and theophylline (7). As compared with other xanthine derivatives, BL 191 is highly soluble in water and the solubility of BL 191 is 10 times that of caffeine and 4 times that of theophylline. Therefore, in view of the high solubility, BL 191 appears to be quite appropriate for the investigation of the effect of xanthine derivatives on the enzyme activity in various organs *in vitro*.

The present paper describes the inhibitory effect of BL 191 on PDE activity in various tissues of the rat.

Sprague-Dawley male rats (CLEA Japan, Inc.) weighing 180-250 g were used in all experiments. The rats were sacrificed by decapitation, and the cerebral cortex, fat pad, ileum, heart muscle and liver were immediately removed. Tissues of each organ were homogenized in icecold 0.32 M sucrose solution containing 50 mM Tris-HCl buffer (pH 7.4) and according to a conventional method, $105000\times g$ supernate was obtained as an enzyme preparation. PDE activity was determined using $360\text{ }\mu\text{M}$ ^{14}C -cyclic AMP ($0.2\text{ }\mu\text{Ci}$) as the substrate according to a modification (7) of the method of Pösch and Kukovetz (8), unless otherwise indicated. Incubation was carried out at 37°C for 15 min (cerebral cortex tissues) or for 40 min (other tissues). Protein contents in the tissues were determined by the method of Lowry et al. (9).

As seen from Lineweaver-Burk plots (Fig. 1), BL 191 inhibited the activity of PDE

FIG. 1. Lineweaver-Burk plots of inhibitory effects of BL 191 on PDE activity in rat tissues

Protein contents in the rat cerebral cortex, fat pad, ileum, heart muscle, and liver were 74.3, 382.0, 216.3, 267.0, and 275.0 μg , respectively.

The concentration of cyclic AMP as substrate ranged from $45\text{ }\mu\text{M}$ to $360\text{ }\mu\text{M}$. V : μ moles cyclic AMP hydrolyzed per mg protein per hr, S : the concentration of the substrate (10^{-3} M)

—●—: None, —▲—: BL 191 10^{-3} M , —■—: BL 191 $2\times 10^{-3}\text{ M}$

TABLE 1. Inhibitory effect of BL 191 on PDE activity in rat tissues

Tissues	Km values (mM)	Ki values (mM)	Type of inhibition
Cerebral cortex	0.16	2.9	Noncompetitive
Fat pad	0.23	2.1	Noncompetitive
Ileum	0.29	1.1	Noncompetitive
Heart muscle	0.08	1.8	Competitive
Liver	0.37	2.6	Competitive

PDE activity was assayed as described in the text with the concentration of Cyclic AMP as substrate ranging from 45 μ M to 360 μ M.

Structure of BL 191:

from the cerebral cortex, fat pad and ileum non-competitively, while its inhibitory effect on heart muscle and liver PDE was competitive.

The Km values of BL 191 for PDE are given in Table 1. BL 191 exerted an inhibitory effect on PDE most markedly in the ileum and then in the heart muscle, fat pad, liver, and cerebral cortex, in the order named, with the Ki values being 1.1, 1.8, 2.1, 2.6 and 2.9 mM, respectively.

Thus, BL 191 caused different PDE inhibitions according to tissues from different organs. Similar results have been reported: caffeine and theophylline were competitive inhibitors of the beef heart PDE (2); caffeine inhibited the dog heart enzyme noncompetitively (3); the type of PDE inhibition in the 2000 $\times$ g supernate by papaverine, an antispasmodic, was different from tissue to tissue (8). It is of great interest that the enzyme has different reaction mechanisms with different PDE inhibitors and in different tissue sources.

Jard and Bernard (10) separated 2 kinds of PDE from frog bladder epithelial cells and rat kidney. In addition, Cheung (11) found the existence of a PDE activator in snake venom and brain tissues and succeeded in purifying the activated and nonactivated PDE which differed in molecular weight. Kakiuchi et al. (12) reported on two kinds of PDE in rat brain tissues termed "Ca²⁺ plus Mg²⁺ dependent PDE" and "Ca²⁺ independent PDE".

The above-described reports strongly suggest the diversity of PDE as a multi-enzyme, and we feel that this diversity should be taken into account in estimating the results of this study. The extent of the diversity of PDE is now being investigated by attempting to clarify the difference of the inhibitory effect of BL 191 on the PDE activity in various organs above described by the method of gel filtration column chromatography.

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PARTICIPATION OF CATECHOLAMINE IN THE DIGITOXIGENIN-INDUCED CONTRACTION OF ISOLATED DOG VEINS

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It was reported that cardiac glycosides such as ouabain and acetylstrophanthidin produced contraction of isolated veins (1, 2), but the mechanism of this action is not yet clear. In this experiment, we intended to obtain further information using the isolated portal and lateral saphenous veins of dogs.

The veins were removed from male mongrel dogs, weighing 9–19 kg, under pentobarbital anesthesia. The segments were immediately placed in Krebs-bicarbonate solution. The portal vein was cut longitudinally (10 mm long and 2 mm wide), and the saphenous vein was made to form a helical strip (10 mm long and 3 mm wide). The preparation was suspended in an organ bath under a tension of 0.4 g for the portal vein and of 0.2 g for the saphenous vein. The organ bath contained 10 ml of the same physiologic salt solution aerated with 95% O₂ + 5% CO₂ and was kept at 37°C. The tension of the strip was recorded isometrically on an ink-writing oscillograph through a force displacement transducer (Nihon Kohden SB-1T). Approximately 1 hr after the strip was suspended, 3 × 10⁻⁶ g/ml digitoxigenin was administered.

The agents used were: digitoxigenin, phentolamine mesylate (Regitine, Ciba), phenoxybenzamine HCl, propranolol HCl (Inderal, Sumitomo Chemical), reserpine (Serpasil, Ciba), acetylcholine (ACh), atropine sulfate, mepyramine maleate and tetrodotoxin (Sankyo). Digitoxigenin was prepared with 95% ethanol in concentration of 10⁻³ g/ml. Reserpine, 0.3 mg/kg/day, was injected intraperitoneally twice on successive days.

After the addition of digitoxigenin 3 × 10⁻⁶ g/ml, both veins contracted, but in different patterns. As shown in Fig. 1 A, the portal vein contracted immediately, followed by a slight relaxation. Then again the tension increased gradually and reached the peak 30–40 min after the addition of digitoxigenin. When the preparation was pretreated with 10⁻⁷ g/ml phentolamine 10 min before the addition of digitoxigenin, the sustained tension development