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EFFECT OF A SELECTIVE β_2-ADRENOCEPTOR AGONIST, PROCATEROL, ON TISSUE CYCLIC AMP LEVEL

ITS DETERMINATION AFTER TISSUE FIXATION BY MICROWAVE IRRADIATION

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Abstract—Intravenous injection of procaterol (1 μmole/kg of body wt), a selective β_2-agonist, into conscious rats caused increases in tissue cyclic AMP, with a peak level at 2 min after injection. Two- to 3-fold increases were induced by procaterol in heart and liver, while 6- to 10-fold increases were induced in trachea, lung and skeletal muscle. Procaterol-induced alterations in cyclic AMP levels in trachea and skeletal muscle were characterized by their long duration (over 2 hr). The duration of the procaterol action reflected sustained stimulation of β-adrenoceptor-linked adenylate cyclase, because the tissue level of cyclic AMP subsided rapidly to the base-line level when propranolol (10 μmoles/kg) was injected intravenously immediately after the peak level was obtained with procaterol. 3-Isobutyl-1-methylxanthine (50 μmoles/kg, s.c.), an inhibitor of phosphodiesterase, was very effective in enhancing the procaterol-induced increases in cyclic AMP in trachea, lung and skeletal muscle. Procaterol caused increases in tissue cyclic AMP levels in skeletal muscle, trachea, lung and heart at doses of 1, 10, 10 and 100 nmoles/kg, respectively, in a dose-dependent manner. It was concluded that procaterol was a long-acting and selective β_2-agonist which was effective in increasing tissue cyclic AMP, as well as in increasing bronchodilator and metabolic alterations. Determination of tissue cyclic AMP using rapid tissue fixation by microwave irradiation could serve as a useful means *in vivo* for the interaction of β-agonists or antagonists with β_1- or β_2-adrenoceptors.

The relaxation in tracheal smooth muscle caused by β-adrenoceptor agonists or theophylline is known to be mediated by an increase in tissue cyclic AMP [1–3]. Therefore, it is useful to measure cyclic AMP levels in trachea for the purpose of evaluating the potency of β_2-adrenoceptor agonists. The measurement of cyclic AMP in trachea has been carried out usually with the isolated organ in the dog and the guinea pig *in vitro* [3–5], rather than with intact animals *in vivo*. Recently, Schmidt *et al.* [6, 7] reported that microwave irradiation provides a means of rapid tissue fixation which is suitable for determination of cyclic AMP concentrations in rat brain areas. Application of this method would be promising as a means for obtaining reliable values of the cyclic AMP level in tracheal smooth muscle *in vivo*.

Procaterol has been shown to be a selective β_2-adrenoceptor agonist based on its abilities to cause bronchodilation [8, 9] and to increase blood levels of metabolites [10]. In the present study, we have examined the effect of procaterol on cyclic AMP levels in rat tissues, including trachea *in vivo*, using the microwave method.

MATERIALS AND METHODS

Male albino rats of the Wistar strain, weighing 160–200 g, were used after 18 hr of starvation. Tissue fixation was achieved by microwave irradiation. Conscious rats were exposed to microwaves for 30 sec and then the tissues were excised and frozen in a precooled clamp. After 30 sec of irradiation, no activity of phosphodiesterase was detected in the tissues examined. The microwave source was a commercial oven (Hitachi MRK-1300; power output 1.3 kW, frequency 2450 MHz). The frozen tissue samples were powdered in liquid nitrogen using a porcelain mortar and pestle. The powder was extracted with 10 vol. of 2 mM EDTA acidified with 0.1 N HCl and the extract was boiled for 10 min in a water bath, as recommended by Honma *et al.* [11]. After centrifugation, the supernatant fraction was assayed for cyclic AMP [11] by a sensitive radioimmunoassay technique with the use of a "cyclic AMP assay kit" obtained from Yamasa Shoyu Co., Chiba, Japan. Phosphodiesterase activity was measured according to the method of Hidaka and Shibuya [12].

The drugs used in this study were 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (procaterol, Otsuka), isoproterenol (Sigma), salbutamol (Leiras), trimetoquinol (Tanabe), propranolol (ICI) and 3-isobutyl-1-methylxanthine (IBMX, Aldrich). Other chemicals were of analytical grade from commercial sources.

RESULTS

Changes of tissue cyclic AMP levels due to procaterol-induced β-adrenoceptor stimulation. Intravenous injection of procaterol (1 μmole/kg of body wt) into conscious rats caused increases in cyclic AMP levels in the trachea, heart, liver and skeletal muscle (Fig. 1). The peak level was observed 2 min after injection, at which time there were 2- to 3-fold increases in heart and liver and 6- to 8-fold increases in trachea and skeletal

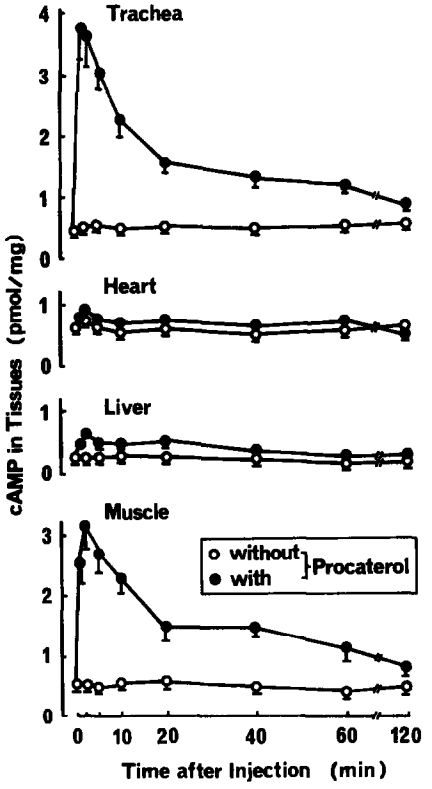

Fig. 1. Effect of procaterol on tissue cyclic AMP levels. Saline (○) or procaterol (●, 1 μmole/kg) was injected intravenously into the tail vein of conscious rats at time 0 and the rats were killed by microwave irradiation at the times shown on the abscissa. Each point represents the mean value from six observations with the S.E.M. shown as a vertical line.

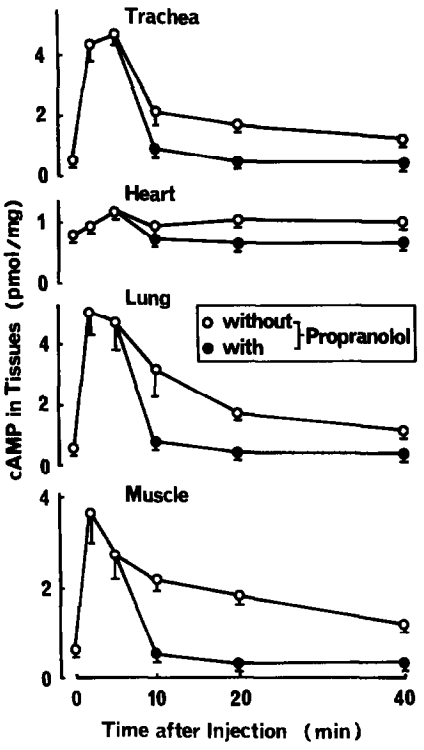

Fig. 2. Inhibition by propranolol of procaterol-induced increases in tissue cyclic AMP levels. Saline (○) or propranolol (●, 10 μmoles/kg) was injected intravenously 5 min after the intravenous injection of procaterol at 1 μmole/kg. Procaterol was injected at time 0. The number of observations is six.

muscle. These peak levels differed significantly ($P < 0.01$ for liver, trachea and skeletal muscle and $P < 0.05$ for heart) from the base-line levels. It should be noted that procaterol-induced alterations in cyclic AMP levels in the trachea and skeletal muscle were so durable as to be observed even at 120 min after injection ($P < 0.05$ for the difference from the preinjection level). Other β-agonists (isoproterenol, trimetoquinol and salbutamol) at a dose of 1 μmole/kg (i.v.) caused roughly 8- ($P < 0.01$), 8- ($P < 0.01$) and 3-fold ($P < 0.05$) increases, respectively, in tracheal cyclic AMP 2 min after the injection (data not shown). These increases, however, were of a much shorter duration; no significant changes were observed after 60 min.

Figure 2 shows the inhibitory effect of propranolol, a β-adrenoceptor antagonist, on the procaterol-induced increase in tissue cyclic AMP. The experiments in Fig. 2 were carried out to examine whether or not the long duration of the procaterol action really reflected sustained stimulation of β-adrenoceptors. Procaterol caused a 10-fold ($P < 0.01$) increase in the lung content of cyclic AMP. Propranolol injected at 5 min after procaterol produced a rapid decline of the concentrations of cyclic AMP in the trachea, heart, lung and muscle. As a result, there were no procaterol-induced increases in cyclic AMP after propranolol, suggesting that the durable action of procaterol was due to stimulation of β-adrenoceptors.

Figure 3 shows the effect of 3-isobutyl-1-methylxanthine (IBMX), a potent inhibitor of cyclic nucleotide phosphodiesterase, on procaterol-induced increases in tissue cyclic AMP. Intravenous injection of procaterol at a dose of 0.1 μmole/kg (just as at 1 μmole/kg) caused increases in tissue cyclic AMP, with the peak level at 2 min after injection. The increase was 1.5-fold ($P < 0.05$) in the heart and 5- to 6-fold ($P < 0.01$) in the trachea, lung and skeletal muscle. Subcutaneous injection of IBMX (50 μmoles/kg) 20 min before procaterol not only produced 2-fold ($P < 0.05$) increases in cyclic AMP levels in these tissues, but also enhanced the procaterol-induced increases in cyclic AMP levels in trachea, lung and skeletal muscle.

Dose-dependent action of procaterol on tissue cyclic AMP levels. The tissue concentrations of cyclic AMP at 2 min after procaterol were plotted as a function of the doses of the drug in Fig. 4. Procaterol, at a dose as low as 10 nmoles/kg (3.4 μg/kg), was effective in the trachea, lung and muscle. The maximal response was induced by 1 μmole/kg of the drug in these tissues. The stimulatory action of procaterol was compared with that of isoproterenol, a non selective β-adrenoceptor agonist (Table 1). Isoproterenol (1 μmole/kg)-induced increases in cyclic AMP levels in the trachea, lung and skeletal muscle were nearly equal to those induced by procaterol. In the heart, isoproterenol produced a greater increase in the cyclic AMP level than did procaterol.

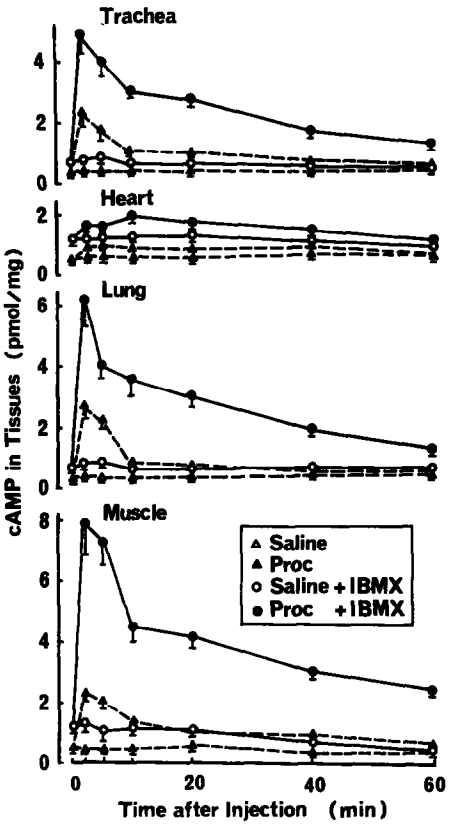

Fig. 3. Effect of IBMX on tissue cyclic AMP levels in the presence or absence of procaterol (Proc). IBMX (50 μmoles/kg) was injected subcutaneously 20 min before the intravenous injection of procaterol at 0.1 μmole/kg. Procaterol was injected at time 0. The number of observations is six.

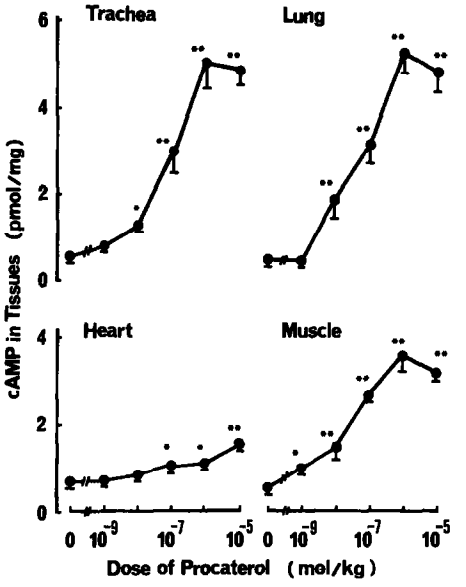

Fig. 4. Dose-dependent increases in tissue cyclic AMP levels after the injection of procaterol. Tissues were fixed by microwave irradiation 2 min after the intravenous injection of procaterol. The significant level for the difference from the control (without procaterol) is indicated by a single or double asterisk: * = $P < 0.05$; ** = $P < 0.01$. The number of observations is six.

DISCUSSION

It has been reported that β_2-adrenoceptors predominate over β_1-adrenoceptors in the trachea [13, 14], in the lung [15, 16] and in the skeletal muscle [16, 17]. In the present study, procaterol (1 μmole/kg) caused 6- to 10-fold increases in cyclic AMP levels in these tissues, whereas it caused lesser increases in the liver and the heart (Figs. 1 and 2). The minimum effective dose of procaterol needed to increase tissue cyclic AMP was 1, 10, 10 and 100 nmoles/kg for the skeletal muscle, trachea, lung and heart respectively (Fig. 4), and the stimulatory action of procaterol on the cyclic AMP level in the heart was less than that of isoproterenol, a non-selective β-adrenoceptor agonist (Table 1). Consequently, it appears that procaterol-induced increases in cyclic AMP were mediated by β_2-adrenoceptors rather than by β_1-adrenoceptors.

Earlier reports [18, 19] have shown that a rapid increase in tissue cyclic AMP induced by epinephrine is followed by a rapid decline *in vivo*. The intravenous injection of procaterol (1μmole/kg) caused a long-lasting (120 min) increase in tissue cyclic AMP (Fig. 1). It is very likely that the long action of procaterol was due to prolonged stimulation of β-adrenoceptors on cell membranes, because the procaterol-induced increase in tissue cyclic AMP was abolished rapidly by the post-injection of propranolol (Fig. 2). Moreover, the stimulatory action of procaterol on tissue cyclic AMP was enhanced remarkably by IBMX, an inhibitor of phosphodiesterase (Fig. 3), in accord with the view that adenylate cyclase activation via β-adrenoceptors, rather than inhibition of phosphodiesterase, was responsible for the procaterol-induced increase in blood cyclic AMP [10].

Table 1. Effects of procaterol and isoproterenol on tissue cyclic AMP levels*

Agent	Heart	Trachea (pmoles/mg tissue)	Lung	Muscle
Control (saline)	0.83 ± 0.04	0.63 ± 0.06	0.49 ± 0.06	0.84 ± 0.09
Procaterol	1.13 ± 0.08†	5.65 ± 0.60‡	6.22 ± 0.95‡	3.63 ± 0.35‡
Isoproterenol	1.52 ± 0.08‡§	6.50 ± 0.69‡	6.34 ± 0.95‡	3.38 ± 0.34‡

* Tissues were fixed 2 min after the intravenous injection of procaterol or isoproterenol at a dose of 1 μmole/kg respectively. The number of observations is six.

† Difference from control is significant ($P < 0.05$).

§ Difference from the procaterol treatment is significant ($P < 0.05$).

‡ Difference from control is significant ($P < 0.01$).

It is unclear to what extent cyclic nucleotide phosphodiesterase contributes to the regulation of the intracellular cyclic AMP level *in vivo*. Subcutaneous injection of IBMX (50 μmoles/kg), which was much more effective than theophylline in increasing blood cyclic AMP (unpublished data), caused roughly a 2-fold increase in tissue cyclic AMP levels (Fig. 3). This action of IBMX was of a much smaller magnitude than the action of procaterol, suggesting that regulation of adenylate cyclase is more responsible than regulation of phosphodiesterase in determining the tissue concentration of cyclic AMP.

In a recent study [10], we measured the blood level of several metabolites, including cyclic AMP, after injection of procaterol into rats. Based on these results, we were led to the conclusion that procaterol is a β_2-adrenoceptor agonist, unique in its selectivity and duration of action. This conclusion was confirmed by the present results obtained with tissue cyclic AMP, as will be discussed below.

First, procaterol caused significant increases in blood lactate [10] and in the cyclic AMP content of skeletal muscle, at a dose as low as 1 nmole/kg (Fig. 4). Since skeletal muscle is probably the main organ liberating lactate into the blood stream, it is very likely that the increase in blood lactate is mediated by β_2-adrenoceptors involved in the generation of cyclic AMP in muscle.

Second, the action of procaterol (1 μmole/kg, i.v.) was prolonged over 2 hr when increases in the tissue cyclic AMP (Figs. 1 and 2) or increases in blood concentrations of glucose, lactate, glycerol, insulin and cyclic AMP [10] were measured as indices of its action. Moreover, the procaterol-induced increase in tissue cyclic AMP occurred in advance of alterations in these blood metabolic parameters. Thus, alterations of these metabolic parameters must reflect β-agonist-induced changes in the tissue level of cyclic AMP.

Application of microwave irradiation as the tissue fixation method made it possible to measure tissue concentration of cyclic AMP *in vivo*, a good index of β-adrenoceptor responses to an administered agent. In the previous paper [20], we reported that the blood level of cyclic AMP can serve as a good index *in vivo* for interaction of agonists or antagonists with β-adrenoceptors. A change in the blood cyclic AMP was readily followed at frequent intervals in one rat, and was found to be much larger in magnitude than the change in tissue cyclic AMP when the rat was injected with β-adrenoceptor agents. Since blood cyclic AMP originates from many tissues, however, it should reflect stimulation of both β_1- and β_2-adrenoceptors. In this regard, the determination of cyclic AMP in a particular tissue is advantageous in that it may reflect the selective stimulation of β_1- or β_2 adrenoceptors by the agonist.

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