EFFECT OF ADRENERGIC AGONISTS ON THE CYCLIC AMP LEVEL IN THE LIVER AND HEART FROM NORMAL AND HYPOTHYROID RATS

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Abstract—In order to clarify the mechanism of the different sensitivity of the adrenoceptors between normal and hypothyroid rats, cyclic AMP levels in the liver and heart were measured after the administration of phenylephrine, isoproterenol, epinephrine and methoxamine. Cyclic AMP increased in all cases, but the extent of its increment in the heart was much less than that in the liver. Phentolamine and propranolol showed only a partial inhibition of cyclic AMP elevation by the agonists in the liver from normal and hypothyroid rat. On the other hand, propranolol blocked completely the effect of the agonists on the heart from both groups. It was also observed that cyclic AMP increased in adrenalectomized rats after the injection of the adrenergic agonists. The basal activity of protein kinase in hypothyroid status was slightly lower than that in the normal, but this enzyme was stimulated in the presence of cyclic AMP in vitro. These results suggest that the function of $\beta$-adrenoceptor remained normal even in hypothyroidism and responded well to isoproterenol and epinephrine. It is also indicated that the increased sensitivity of $\alpha$-adrenoceptor to phenylephrine in the hypothyroid atria previously observed is probably in part independent of the mechanism mediated by cyclic AMP.

It is well known that the effects of catecholamines on several tissues are mediated by intracellular cyclic AMP (1) and also regulated by thyroid status (2).

In previous studies we have shown the increased sensitivity of $\alpha$-adrenoceptor in the atria of hypothyroid rats and the reduced sensitivity of $\beta$-adrenoceptor in the atria and the liver of the same animals (3-7). Namely, positive inotropic and chronotropic responses of the rat atria to phenylephrine were increased in hypothyroid status. On the other hand, the stimulative effect of isoproterenol on the atria was reduced under that condition. Furthermore, increased gluconeogenesis from lactate could still be observed in the hypothyroid liver by the administration of phenylephrine, whereas that effect of isoproterenol disappeared.

In order to clarify whether or not these phenomena in hypothyroid status are dependent on changes in levels of cyclic AMP, these levels in the liver and heart were determined after the injection of the adrenergic agonists. Protein kinase activity was also determined in these tissues.

MATERIALS AND METHODS

Male Wistar strain rats were obtained from the Eisai Biological Institute and were used

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throughout this study. Rats were made hypothyroid by providing a 0.15% propylthiouracil diet for approx. 12 weeks. Adrenalectomized rats were given 0.9% NaCl as drinking water for 5 to 7 days, after which they were sacrificed. L-Phenylephrine hydrochloride (PHE) was purchased from Kohwa Chemicals, L-isoproterenol hydrochloride (ISP) from Kaken Chemicals, L-epinephrine hydrochloride (EPI) from Daiichi Seiyaku and L-methoxamine hydrochloride (MTX) from Nihon Shinyaku. Adenosine 3',5'-monophosphate (cyclic AMP) was obtained from C. F. Boehringer Mannheim and histone (type IIA) from Sigma. Cyclic [3H] AMP (30 Ci/mmol) was a product of New England Nuclear. \([\gamma^{32P}]\) ATP was kindly donated by Dr. M. Kobayashi (Dept. of Biochem., Nagoya Univ. School of Med.). Other chemicals were obtained from commercial sources.

The following two doses of each agonist were applied: phenylephrine, 0.04 and 0.3 mg/kg; isoproterenol, 0.002 and 0.2 mg/kg; epinephrine, 0.01 and 0.2 mg/kg; methoxamine, 0.04 and 0.4 mg/kg. Adrenergic agonists were administered i.p. and the animals were sacrificed at various time periods for the determination of cyclic AMP in the liver and heart. When adrenergic blocking agents were used with the agonist, 1 mg/kg of the blocker was injected i.p. 10 min before the administration of the agonist.

Cyclic AMP was measured by isotope dilution using a cyclic AMP-binding protein obtained from the rabbit skeletal muscle according to Gilman (8). The values of the figures at each time period represent the mean of 3 to 4 tissues.

The assay of protein kinase was carried out by the method described by Walsh et al. with slight modification (9).

RESULTS

Changes in cyclic AMP level in the liver

The basal cyclic AMP levels in livers from normal and hypothyroid rats were 1.39 ± 0.18 nmoles/g wet weight (mean ± S.D., n=3) and 1.16±0.21 nmoles/g wet weight (n=4), respectively. When large doses of phenylephrine, isoproterenol and epinephrine were applied, the cyclic AMP level increased by approx. 2-fold within 1 min and returned to the basal level at 5 min in normal rats (Fig. 1, bottom). In small doses, increase in cyclic AMP level was also observed, but the extent was less than in large doses (Fig. 1, top). Changes in the levels of cyclic AMP in the hypothyroid liver were practically the same as observed in the normal in both small and large doses (Fig. 1).

Changes in cyclic AMP level in the heart

As shown in Fig. 2, the basal levels of cyclic AMP in normal and hypothyroid rat hearts were 1.57±0.20 nmoles/g wet weight (mean ± S.D., n=3) and 1.36±0.12 nmoles/g wet weight (n=4), respectively. The level in the heart reached a maximum within 2 min and maintained this level for 5 min in all cases after the administration of the agonists.

Effect of methoxamine on cyclic AMP level

Since the data above described showed that phenylephrine increased the cyclic AMP level in both tissues, two doses of methoxamine were administered as another α-adrenergic
agonist. Fig. 3 shows that the levels of cyclic AMP in two tissues from normal and hypothyroid rats increased with the injection of this drug. However, changes in the level in the heart were considerably less as compared with those in the liver with either a small or large dose.

Effects of phentolamine and propranolol on the elevation of cyclic AMP level by adrenergic agonists

As shown in Table 1, phentolamine blocked the increase in cyclic AMP level by phenylephrine in the liver. However, only a partial inhibition was observed in cases of other agonists. In the heart, phentolamine also showed a partial inhibition to all agonists. On the contrary, propranolol caused only a partial inhibition of the increase in cyclic AMP level in the liver, whereas complete inhibition in the heart from both normal and hypothyroid rats was seen with this drug.

Effect of adrenalectomy on cyclic AMP level in the liver and heart

The present results indicate that all adrenergic agonists are able to increase the cyclic AMP level in the liver and heart from both normal and hypothyroid rats. Accordingly, in
FIG. 2. Cyclic AMP level in the hearts of normal and hypothyroid rats after administration of phenylephrine, isoproterenol and epinephrine. See legend to Fig. 1 for other information.

Fig. 3. Changes in cyclic AMP levels in the liver and hearts after the injection of methoxamine. Small dose: 0.04 mg/kg i.p. Large dose: 0.4 mg/kg i.p.
**ADRENERGIC AGONISTS AND CYCLIC AMP**

**Table 1.** Effects of phentolamine and propranolol on increase in cyclic AMP level by the agonists

|          | Liver N* | H* | Heart N* | H* |
|----------|----------|----|----------|----|
| None     | 1.39     | 1.16 | 1.57     | 1.36 |
|          | ±0.18    | ±0.21 | ±0.19    | ±0.12 |
| Phenylephrine | 3.12     | 1.77 | 2.08     | 1.93 |
|          | ±1.01    | ±0.02 | ±0.15    | ±0.01 |
| +Phentolamine | 1.60     | 1.36 | 2.16     | 1.88 |
|          | ±0.15    | ±0.15 | ±0.69    | ±0.12 |
| +Propranolol | 1.67     | 1.61 | 1.41     | 1.34 |
|          | ±0.33    | ±0.14 | ±0.14    | ±0.37 |
| Isoproterenol | 3.11     | 2.87 | 2.28     | 2.23 |
|          | ±0.44    | ±0.32 | ±0.12    | ±0.31 |
| +Phentolamine | 2.24     | 2.32 | 2.66     | 1.63 |
|          | ±0.17    | ±0.81 | ±0.18    | ±0.23 |
| +Propranolol | 2.03     | 2.20 | 1.43     | 1.46 |
|          | ±0.26    | ±0.40 | ±0.25    | ±0.28 |
| Epinephrine | 2.61     | 3.77 | 2.26     | 2.23 |
|          | ±0.64    | ±1.78 | ±0.27    | ±0.51 |
| +Phentolamine | 2.30     | 1.92 | 2.05     | 1.71 |
|          | ±0.38    | ±0.79 | ±0.20    | ±0.50 |
| +Propranolol | 2.02     | 2.32 | 1.38     | 1.59 |
|          | ±0.36    | ±0.56 | ±0.39    | ±0.22 |

* N=normal rats, H=hypothyroid rats.

**Table 2.** Effect of adrenalectomy on cyclic AMP level in the liver and heart

|          | Liver Eu* | H* | Heart Eu* | H* |
|----------|----------|----|-----------|----|
| None     | 1.32     | 1.47 | 1.47      | 1.46 |
|          | ±0.20    | ±0.03 | ±0.41    | ±0.18 |
| Phenylephrine | 2.99     | 1.67 | 2.27     | 1.85 |
|          | ±0.44    | ±0.14 | ±0.13    | ±0.07 |
| Isoproterenol | 3.33     | 5.28 | 2.60     | 2.13 |
|          | ±0.39    | ±1.23 | ±0.28    | ±0.13 |
| Epinephrine | 3.08     | 1.98 | 2.26     | 1.77 |
|          | ±0.08    | ±0.06 | ±0.05    | ±0.35 |

* Eu= euthyroid rats, H=hypothyroid rats.

In order to demonstrate that these increased levels of cyclic AMP are not dependent on the endogenous catecholamines, cyclic AMP level was measured in adrenalectomized rats. In this experiment, large doses of the agonists were administered and the rats were sacrificed 1.0 min after the injection. As shown in Table 2, there was no difference in the basal level between the two groups, and in adrenalectomized rats, essentially the same change as that
in the control animals was observed after the administration of the agonists. The same results were also observed in hearts from adrenalectomized rats.

**Protein kinase activities of livers and hearts from normal and hypothyroid rats**

Protein kinase activities of livers and hearts were measured in the absence and in the presence of cyclic AMP. As shown in Table 3, the basal activity of this enzyme in hypothyroid status was somewhat lower than that in normal, that is, 41.6 and 32.3 pmoles $^{32}$P incorporated/mg/min in the liver and 52.6 and 45.8 pmoles $^{32}$P incorporated/mg/min in the heart, respectively. When 2.0 $\mu$M cyclic AMP was added to the reaction mixture, the enzyme activity was stimulated by approx. 2.5-fold in all groups.

**Table 3. Protein kinase activity of the liver and heart in the absence and in the presence of cyclic AMP**

|          | Normal | Hypothyroid |
|----------|--------|-------------|
|          | Cyclic AMP | Cyclic AMP |
|          | (−) | (+) | (−) | (+) |
| pmoles $^{32}$P incorp./mg/min |
| Liver    | 41.6 | 98.1 | 32.3 | 79.5 |
| Heart    | 52.6 | 134.9 | 45.8 | 141.8 |

**DISCUSSION**

In previous studies in this laboratory, it was found that the dose response curve for the positive inotropic action of phenylephrine shifted to the left in the hypothyroid rat atria, whereas that of isoproterenol shifted to the right (3, 5). Recently, this phenomenon was confirmed in other laboratories (10, 11). These data suggested two possibilities for the explanation of the increased sensitivity of hypothyroid rat heart to phenylephrine; firstly, there are two adrenoceptors, $\alpha$ and $\beta$, in the rat heart, and while the effect of phenylephrine is mediated through $\alpha$-adrenoceptor, this effect is enhanced under hypothyroidism; secondly, the receptor in the heart is only a $\beta$-type receptor and its property changes from $\beta$ to $\alpha$ according to changes in metabolism or environment. Furthermore, the decreased sensitivity to isoproterenol in hypothyroid rat hearts and livers may be due to the change in the property of $\beta$-adrenoceptor followed by reduced level in cyclic AMP.

The present study has demonstrated that the basal level of cyclic AMP was essentially the same both in normal and hypothyroidism, and that the level of cyclic AMP increased in the hearts from both groups with the injection of adrenergic agonists. This increased level in cyclic AMP was completely blocked by the pretreatment with propranolol in hearts from both groups (Table 1). On the other hand, increased positive inotropic response to phenylephrine in hypothyroid rat atria was blocked by phentolamine as previously reported (3). So, there is a dissociation of catecholamine-induced positive inotropic effect from an increased cyclic AMP level, the former being induced through $\alpha$-adrenoceptor, and the latter through $\beta$-adrenoceptor. Recently, similar results were obtained by Osnes and Øye in the normal rat heart (11). Furthermore, Schüman et al. reported that isoproterenol
caused an elevation in the cyclic AMP level and then stimulated positive inotropic action in rabbit papillary muscle, whereas methoxamine increased the positive inotropic response without the increment of cyclic AMP level (12). Henry et al. and Venter et al. also reported the dissociations between changes in cyclic AMP level and contractility in guinea pig heart (13) and in cat papillary muscle (14), respectively.

Taking into account the present results together with those reports above described, the increased inotropic response of the hypothyroid rat heart to phenylephrine can be interpreted by the first possibility: the effect of phenylephrine is mediated by an unknown pathway independent of cyclic AMP through α-adrenoceptor and this effect is enhanced under hypothyroid status.

The changes in cyclic AMP levels cannot explain the decreased sensitivity of β-adrenoceptor, such as decreased gluconeogenic rate in the liver and the decreased inotropic response in the heart to isoproterenol in hypothyroidism, as the cyclic AMP levels increased in both normal and hypothyroid rats with the administration of phenylephrine, isoproterenol, epinephrine or methoxamine. Interesting observations of Armstrong et al. (15, 16), however, suggest another possibility that the enzymes related to cyclic AMP metabolism play an important role on the function through the β-adrenoceptor. Their studies showed that lipolytic response to epinephrine was blocked in fat cells from hypothyroid rats. Adenylate cyclase was activated by epinephrine even in hypothyroidism, but the activity of cyclic AMP phosphodiesterase was elevated and thus cyclic AMP was rapidly degraded into 5'-AMP by this enzyme under that condition. Consequently, the amounts of cyclic AMP available for the stimulation of lipolysis are reduced in fat cells. Moreover, Katz et al. demonstrated the relationship between calcium transport and protein kinase in cardiac sarcoplasmic reticulum (17–19). Therefore, in order to clarify the mechanism through β-adrenoceptor, it is necessary to investigate the changes in the properties of these enzymes in the heart and liver from hypothyroid rats. Such studies are now in progress.

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