# SELECTIVE REPRESSION OF BENZOATE OR TRYPTOPHAN MEDIATED INDUCTION OF LIVER TYROSINE AMINOTRANSFERASE BY PHENTOLAMINE IN ADRENALECTOMIZED RATS

K.KATO

*Department of Biochemistry, School of Medicine, University of Nagoya, Nagoya, Japan*

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## 1. Introduction

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is postulated as an intracellular mediater for the control of several enzymes forming systems [1, 2]. Induction of rat liver tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) by glucagon [3], epinephrine [4] and cyclic AMP [4, 5] has been shown in adrenalectomized animals, in perfused liver and in fetal liver. Epinephrine induced increase of the enzyme activity in fetal liver is blocked by dichloroisoproterenol, an adrenergic blocking agent [4].

Phentolamine (2-[*N*-(*m*-hydroxyphenyl)-*p*-toluidinomethyl] imidazole), an adrenergic blocking agent, is reported to be an inducer of liver tyrosine aminotransferase in intact rats, but the induction by this agent is completely abolished by either adrenalectomy or hypophysectomy [6].

During a study of the control mechanism of liver tyrosine aminotransferase, *in vivo*, by non-hormonal substances, it was found that phentolamine selectively blocked the benzoate or tryptophan mediated induction of this enzyme. These results are presented in this report.

## 2. Experimental

Adrenalectomized male Wister rats of 130 to 170 g body weight were fasted overnight before the experiments began. They were given 0.9% NaCl as drinking water at all times. Sodium benzoate, hydrocortisone succinate (Solu-Cortef, Upjohn) or glucagon (Novo) was injected intraperitoneally. L-Tryptophan was administered in 2 ml of 0.9% NaCl by stomach tube. Rats were killed between 1 to 3 p.m. Tyrosine aminotransferase was assayed with liver homogenate according to the method of Kenney [7] with modifications. *p*-Hydroxyphenylpyruvate formed was determined by a modification of the Briggs method [8]. Tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) was assayed with the 6000 *g* supernatant fraction of liver homogenate according to the method of Feigelson and Greengard [9]. Protein concentrations were determined by the biuret procedure.

## 3. Results

Administration of sodium benzoate to adrenalelectomized rats results in a several fold increase in the activity of liver tyrosine aminotransferase by a process sensitive to puromycin and actinomycin D [10]. As seen in table 1 and fig. 1, the induction of tyrosine aminotransferase by benzoate was repressed by the administration of phentolamine. Phentolamine alone did not change the basal level of the enzyme in adrenalectomized rats, as previously reported by Govier and Lovenberg [6]. The addition of phentolamine to the assay system up to $3 \times 10^{-4}$ M showed no effect on the enzyme activity. Among the several adrenergic blocking agents tested, phenoxybenzamine (dibenzylline) repressed the induction as effectively as phentolamine, but ergotamine and propranolol showed no effect.



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Table 1
Effect of phentolamine on the induction of tyrosine aminotransferase in adrenalectomized rats.

| Treatment | Period of induction (hr) | No. of animals | TAT activity (mμmoles/min/mg) | TP activity (mμmoles/hr/mg) |
|---|---|---|---|---|
| None | | 14 | 16.9 ± 2.8 | |
| Phentolamine (3 mg) | 3 | 7 | 14.1 ± 2.0 | |
| Benzoate (30 mg) | 3 | 6 | 50.7 ± 3.1 | |
| Benzoate + phentolamine (3 mg) | 3 | 4 | 23.3 ± 3.5 | |
| Benzoate + phenoxybenzamine (2 mg) | 3 | 4 | 21.1 ± 2.0 | |
| Benzoate + ergotamine (1 mg) | 2 | 2 | 42.0 | |
| Benzoate + propranolol (1 mg) | 3 | 3 | 42.8 ± 3.6 | |
| None | | 4 | 19.0 ± 3.7 | 26.3 ± 5.1 |
| Phentolamine* (7 mg) | 4 | 4 | 16.7 ± 1.2 | 29.6 ± 3.1 |
| Glucagon (0.5 mg) | 3 | 3 | 40.4 ± 3.9 | |
| Glucagon + phentolamine* (5 mg) | 3 | 4 | 39.8 ± 4.2 | |
| Hydrocortisone (10 mg) | 4 | 5 | 114.4 ± 10.0 | 161.3 ± 21.3 |
| Hydrocortisone + phentolamine* (7 mg) | 4 | 5 | 124.8 ± 3.0 | 135.5 ± 13.3 |
| Tryptophan (100 mg) | 4 | 5 | 64.2 ± 8.2 | 91.2 ± 10.7 |
| Tryptophan + phentolamine* (7 mg) | 4 | 5 | 30.5 ± 1.9 | 94.0 ± 11.3 |

The rats weighing 130 to 170 g were fasted overnight. The indicated doses of sodium benzoate, glucagon, hydrocortisone succinate or L-tryptophan were administered as described in Experimental. Phentolamine (Regitine, CIBA), ergotamine (Sigma), phenoxybenzamine (dissolved in 50% ethanol) or propranolol (Inderal) was injected intraperitoneally. Tyrosine aminotransferase (TAT) activity is expressed as mμmoles *p*-hydroxyphenylpyruvate formed/min/mg protein and tryptophan pyrrolase (TP) activity, as mμmoles kynurenine formed/hr/mg protein. Each value is the average ± standard deviation of the mean.

* Phentolamine was given at 0 (3 mg), 1.5 (2 mg) and 3 hr (2 mg) after the inducer.

It might be argued that phentolamine or phenoxybenzamine depresses the synthesis of all proteins in the liver to an equal degree, and that the repressive effect of these agents is seen on another inducing processes of liver enzymes. To examine this possibility, the effect of phentolamine on the induction of tyrosine aminotransferase and tryptophan pyrrolase by other inducers was tested. As shown in table 1, induction of these enzymes by glucagon or hydrocortisone was not affected even by the repeated administration of phentolamine. However, induction of tyrosine aminotransferase by tryptophan [11] was repressed under the condition in which the induction of tryptophan pyrrolase was not affected.

These results indicate that the repressive effect of phentolamine is selective and does not result from the depression of the general protein synthesizing process in the liver nor from the disturbance of the absorption or circulation of the inducer.

## 4. Discussion

Although the increased activity of tyrosine aminotransferase appears to result from the *de novo* synthesis of enzyme protein, it is not clear whether the administered benzoate or tryptophan acts directly on the liver or stimulates the secretion of some hormone which brings about the induction of this enzyme in the liver. However, present results indicate that enzyme induction by benzoate or tryptophan is selectively repressed by phentolamine.

It is proposed that the adrenergic blocking agents block the physiological responses of hormones by inhibiting the hormone mediated accumulation of cyclic AMP [12–14]. From this concept and the present results, it is suggested that the mechanism of benzoate or tryptophan mediated induction of tyrosine aminotransferase includes a cyclic AMP accumulating process, which is sensitive to phentolamine and

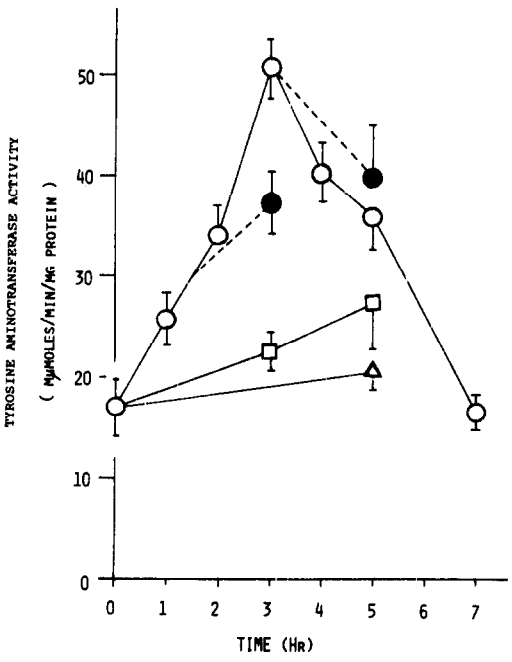


Fig. 1. Time course of induction of tyrosine aminotransferase by benzoate and its repression by phentolamine in adrenalectomized rats. The rats were given the following treatment (intraperitoneal injection):
○——○, sodium benzoate (130 mg/150 g body weight)
□——□, sodium benzoate + phentolamine (3 mg/150 g body weight)
△——△, sodium benzoate + phentolamine (3, 2 and 2 mg at 0, 1.5 and 3 hr, respectively)
●- - -●, sodium benzoate + phentolamine (3 mg at 1.5 or 3 hr after benzoate).

Each point is the average of 4 to 7 animals. Vertical bars indicate standard deviation of the mean.

phenoxybenzamine. However, it remains as a possible mechanism that phentolamine or phenoxybenzamine stimulates the release of some endogenous repressive substance, or forms a complex with the enzyme protein or pyridoxal phosphate *in vivo* as suggested in the case of norepinephrine [15].

## References

[1] R.L.Perlman, B.D.Crombrugghe and I.Pastan, Nature 223 (1969) 810.
[2] J.P.Post, A.W.Hsie and H.V.Rickenberg, Biochem. Biophys. Res. Commun. 34 (1969) 748.
[3] O.Greengard and G.T.Baker, Science 154 (1966) 1461.
[4] W.D.Wicks, Science 160 (1968) 997.
[5] W.D.Wicks, F.T.Kenney and K.L.Lee, J. Biol. Chem. 244 (1969) 6008.
[6] W.C.Govier and W.Lovenberg, Biochem. Pharmacol. 18 (1969) 2667.
[7] F.T.Kenney, J. Biol. Chem. 234 (1959) 2707.
[8] Z.N.Canellakis and P.P.Cohen, J. Biol. Chem. 22 (1956) 53.
[9] P.Feigelson and O.Greengard, J. Biol. Chem. 236 (1961) 153.
[10] S.Singer and M.Mason, Biochim. Biophys. Acta 110 (1965) 370.
[11] F.Labrie and A.Korner, J. Biol. Chem. 243 (1968) 1116.
[12] G.A.Robinson, R.W.Butcher and E.W.Sutherland, Ann. N.Y. Acad. Sci. 139 (1967) 703.
[13] S.E.Mayer, B.J.Williams and J.M.Smith, Ann. N. Y. Acad. Sci. 139 (1967) 686.
[14] G.S.Levey, J.Roth and I.Pastan, Endocrinology 84 (1969) 1009.
[15] I.B.Black and J.Axelrod, J. Biol. Chem. 244 (1969) 6124.