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NATURE OF THE INHIBITION BY NORADRENALINE OF INDUCTION BY CORTISOL OF HEPATIC TRYPTOPHAN PYRROLASE

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Abstract—Administration of noradrenaline inhibited the induction of hepatic trytophan pyrrolase by cortisol but not by tryptophan. The selective inhibition of pyrrolase was specific to noradrenaline, whereas adrenaline and rat growth hormone also inhibited tyrosine aminotransferase. None of those three hormones had any effect on the incorporation of [^{32}P]-orthophosphate into RNA, stimulated by cortisol. Other biogenic amines, polypeptide hormones and steroid analogues were not inhibitory to the induction of tryptophan pyrrolase by cortisol. The α-adrenergic agonist, phenylephrine, potentiated the noradrenaline inhibition whereas DL-threo-3,4-dihydroxyphenylserine, its precursor, together with pargyline had no effect on the induction process of pyrrolase. These results support the view that noradrenaline exerts its inhibitory action at the cell membrane via the α-receptor, and is not mediated directly by an intracellular mechanism.

The two hepatic enzymes, tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.1.12) and tyrosine aminotransferase (EC 2.6.1.5), are known to be induced by treatment of rats with exogenous corticosteroids or by stress conditions in which their circulating concentrations are increased. A selective inhibition of the induction of tryptophan pyrrolase alone was observed under certain conditions such as cold exposure, hypobaric hypoxia and treatment with bacterial pyrogens [1–4]. Treatment with noradrenaline produced a similar selective inhibition of tryptophan pyrrolase in the early phase of induction by cortisol [5]. This system gave an experimental model for regulation of an enzyme by unique interaction of these two "stress hormones".

The second messenger hypothesis of Sutherland, wherein a variety of hormones interact with a plasma membrane receptor leading to activation of adenylate cyclase, resulting in specific hormonal responses, has been extended to several hormones. Catecholamines are known to interact with specific populations of receptors on the plasma membrane, the α- and the β-receptors, which are distinguished by the blockade of discrete effects of these hormones by receptor specific agents. Of these, only activation of β-receptors results in accumulation of cyclic AMP. The classical studies of Sutherland on the livers of cat, dog and rabbit led to the finding that the action of catecholamines on glycogenolysis is dependent on the β-receptor-cyclic AMP system [6]. However, recent studies with rat liver have shown the presence of an additional α-receptor-dependent mechanism for glycogenolysis [7, 8]. It is increasingly being recognized that catecholamines can interact with both populations of receptors in producing enzyme changes.

It was shown in this laboratory that the above described selective inhibition by noradrenaline could be reversed by simultaneous treatment with α-adrenergic blocking agents and not with β-blockers [9]. The present investigation focussed attention on this induction inhibition-reversal system with respect to the following: the possible competition of other steroids with cortisol in the induction process, the effect of a variety of amines and hormones in substituting for noradrenaline in the inhibition process, the effect of adrenergic agonists which potentiate the action of catecholamines, and the effect of noradrenaline on ^{32}P-incorporation into RNA, stimulated by cortisol.

MATERIALS AND METHODS

Chemicals. The compounds used in the experiments were obtained from the following sources: L-tryptophan, L-tyrosine, DL-threodihydroxy-phenylserine, serotonin creatine sulfate, 5-hydroxytryptophan, 3,4-dihydroxyphenylethylamine, theophylline, cortisol, corticosterone, isoproterenol (*dl*-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol) and colchicine from Sigma Chemical Co., St. Louis, MO, U.S.A.; noradrenaline, adrenaline, L-dihydroxyphenylalanine, acetylcholine and histamine from Koch–Light Laboratories, Colnbrook, Bucks, U.K.; pargyline hydrochloride (*N*-benzyl-*N*-methylprop-2-ynylamine hydrochloride) from Abbot Co., North Chicago, IL, U.S.A.; D-glucose from Sarabhai–Merck Chemicals India, Baroda, India; follicle stimulating hormone (FSH, rat FSH-B-1), luteinizing hormone (LH, ovine, NIH-LH-S19), prolactin (bovine, NIH-P-B4), thyroid stimulating hormone (TSH, bovine, NIH-TSH-B.6) and growth hormone (rat GH-B-2) from Hormone Distribution Programme, NIAMD, NIH, Bethesda, MD, U.S.A.; glucagon from Eli Lilly Co., Indianapolis, IN, U.S.A.; 17-hydroxyprogesterone and 19-methyltestosterone from Biochemicals Unit, V.P. Chest Institute, Delhi, India; phenoxybenzamine hydrochloride *N*-(2-chloroethyl)-*N*-(1-methyl-2-phenoxyethyl) benzylamine hydrochloride and chloropromazine from Smith, Kline & French, Bangalore, India; phenylephrine (3-hydroxyphenyl-α-methylaminoethanol) from Dr. R. M. Marchbanks, Institute of Psychiatry, London SE5 8AF, U.K.; and carrier-free [^{32}P]-orthophosphate from Bhabha Atomic Research Centre, Bombay, India.

Animals and treatment. Male albino rats of the Wistar strain, weighing 170 ± 20 g, from the Central Animal Facility of the Institute were used. The

animals were fed *ad lib.* on a pellet diet obtained from Hindustan Lever, Bombay, India, containing 24% protein, 4% fat, 50% carbohydrate and other nutrients. Water was given *ad lib.* All the compounds were administered intraperitoneally in 0.9% NaCl, either in solution or in suspension and the control animals received equivalent amounts of 0.9% NaCl. The animals were pretreated with various compounds as stated immediately before injecting the inducer, cortisol (10 mg/rat). The animals were killed normally 3 hr after cortisol treatment by cervical dislocation. All killings were done between 10:00 and 11:00 a.m. to avoid interference of rhythmic variations. The livers were homogenized in 2 vol. (v/w) of cold (0°) 1.15% (w/v) KCl in a Potter–Elvehjem-type glass homogenizer and the homogenates were centrifuged at 36,000 *g* for 40 min in a refrigerated Sorvall RC 2B centrifuge. The supernatant fractions were used for assaying tryptophan pyrrolase after activation (by preincubation with tryptophan) as described by Sitaraman and Ramasarma [4] and tyrosine aminotransferase according to the method of Diamondstone [10] with some modifications adopted by Namboodiri and Ramasarma [11]. Appropriate control groups of animals were killed simultaneously in each experiment and the results were compared with the experimental groups processed the same day, because the control values varied on different days. Each group consisted of six or more rats and values of mean ± S. E. M. were obtained for analyzing the statistical significance by Student's *t*-test. Specific activity of the enzymes is expressed as nmoles of product formed/hr/mg of protein.

All the compounds injected in animals in these experiments were tested separately for their effect on the activities of the enzymes *in vitro* and were found to have no effect in the range of 2–100 μg in the reaction mixtures. In the last set of experiments, the livers were homogenized in a medium consisting of 0.25 M sucrose, 0.03 M potassium phosphate buffer (pH 7.4) and 0.14 M NaCl (10%, w/v) and then made to 0.3% (w/v) with deoxycholate. RNA was isolated from these mixtures by the hot phenol extraction procedure described by Schutz *et al.* [12]. The final RNA samples were dissolved in 2 ml of 0.01 M Tris–HCl buffer (pH 7.2) and the amount of RNA was measured by its absorption at 260 nm in a Beckman DU-2 spectrophotometer. Aliquots of 0.1 ml were counted in a Well-type Beckman model LS-100 liquid scintillation counter in a scintillation mixture containing 0.5% PPO* and 6% naphthalene (w/v) in dioxane with a counting efficiency of 95 per cent.

RESULTS

Specificity of cortisol. Both tryptophan pyrrolase and tyrosine aminotransferase are known to be induced by treatment of rats with cortisol and tryptophan. The inhibition by noradrenaline was found to be specific for the induction of tryptophan pyrrolase obtained by treatment with cortisol but not with tryptophan (Table 1). Tyrosine aminotransferase remained unaffected, confirming the selective inhibition of induction of pyrrolase by noradrenaline.

* PPO = 2,5-diphenyloxazole.

*Table 1. Effect of noradrenaline on enzyme induction by cortisol and tryptophan**

Treatment	Enzyme activity (nmoles/hr/mg protein)	
	Noradrenaline	+ Noradrenaline
Tryptophan pyrrolase		
Cortisol	117 ± 8	72 ± 10†
Tryptophan	60 ± 5	61 ± 5
Tyrosine aminotransferase		
Cortisol	2200 ± 250	1980 ± 360
Tryptophan	1890 ± 130	1980 ± 240

* The first set of two groups of rats was treated intraperitoneally with cortisol (10 mg/rat) and the second set of two groups of animals was treated with tryptophan (100 mg/rat). In each set, one group was given noradrenaline (0.2 mg/rat) and pargyline (5 mg/rat) just prior to the injections of the inducing agents. All the animals were killed 3 hr after the injections. The values are expressed as mean ± S. E. M.

† Significant change obtained on treatment with noradrenaline, $P < 0.01$.

Table 2. Effect of competitive steroids on the induction by cortisol of hepatic tryptophan pyrrolase*

Treatment	Enzyme activity (nmoles/hr/mg protein)	
	−Cortisol	+Cortisol
None	25 ± 5	143 ± 13†
17-Hydroxyprogesterone	94 ± 6†	171 ± 5†
None	21 ± 4	148 ± 17†
19-Methyltestosterone	59 ± 9†	149 ± 19†

* Groups of rats were treated intraperitoneally with cortisol (10 mg/rat), 17-hydroxyprogesterone (10 mg/rat) or 19-methyltestosterone (10 mg/rat) either separately or in combinations as specified. All animals were killed 3 hr after the injections. The values are expressed as mean ± S. E. M.

† Significant change obtained on treatment with steroids compared to control groups without steroids, $P < 0.01$.

Since the effect was limited to cortisol, the possibility that some other steroids released in response to stress may compete with noradrenaline in the process of induction of the enzyme was considered next. For testing this hypothesis, the steroid analogues, 17-hydroxyprogesterone and 19-methyltestosterone, already shown to possess such competitive action with respect to tyrosine aminotransferase in hepatoma cell cultures [13], were chosen. The results in Table 2 show that these compounds had no effect on the cortisol-mediated induction of pyrrolase. Since these were experiments *in vivo*, it is difficult to distinguish the intrinsic action of these compounds from mobilization of endogenous corticosteroids.

Specificity of noradrenaline. In the second set of experiments, the specificity of noradrenaline was tested. A number of compounds, including some amines, along with pargyline, and hormones were used. In order to avoid any alteration in cortisol available for induction as a result of administering these compounds, cortisol was given at a dose (10 mg/rat) larger than that required for maximum induction (5 mg/rat). The compounds were first administered at the specified doses, followed by cortisol, and the

animals were killed after 3 hr. In each experiment, a group treated with cortisol alone served as the control. There was no effect on the induction of the pyrrolase when the animals were treated with the following: 5-hydroxytryptamine (1.5 mg/rat), 5-hydroxytryptophan (5 mg/rat), L-dihydroxyphenylalanine (50 mg/rat), L-dihydroxyphenylethylamine (0.2 mg/rat), histamine (0.2 mg/rat), follicle stimulating hormone (0.6 mg/rat), luteinizing hormone (0.3 and 0.6 mg/rat), thyroid stimulating hormone (0.3 and 0.6 mg/rat), prolactin (0.3 and 0.6 mg/rat), theophylline (15 mg/rat), glucagon (0.2 mg/rat), acetylcholine (3 mg/rat), and colchicine (0.2 mg/rat). The results are not given, as all these compounds had little effect. The significant conclusion from these negative, yet valuable, experiments is that the inhibitory process, as noted above, is specific for noradrenaline. These results, in addition to pointing to the lack of participation of any of these compounds, also exclude that which is affected by them, such as cyclic nucleotides and microtubules.

Table 3. Effect of adrenaline, rat growth hormone and glucose on the induction of the enzymes by cortisol*

Treatment	Enzyme activity (nmoles/hr/mg protein)	
	Cortisol	Cortisol + compound
Tryptophan pyrrolase		
Adrenaline	115 ± 11	68 ± 5†
Rat growth hormone	118 ± 17	79 ± 9‡
Glucose	91 ± 6	65 ± 4†
Tyrosine aminotransferase		
Adrenaline	1250 ± 30	960 ± 20‡
Rat growth hormone	1040 ± 60	830 ± 40‡

* Groups of rats were treated intraperitoneally with adrenaline (0.04 mg/rat), rat growth hormone (0.6 mg/rat) or glucose (200 mg/rat), as specified, just prior to cortisol (10 mg/rat) which was given to all rats. All the animals were killed 3 hr after the injections. The values are expressed as mean ± S. E. M.

† Significant change obtained on treatment with the compounds compared to control groups treated with cortisol alone, $P < 0.01$.

‡ Significant change obtained on treatment with the compounds compared to control groups treated with cortisol alone, $P < 0.05$.

Non-selective inhibition of the two enzymes by adrenaline. The inhibition of induction by cortisol of both tryptophan pyrrolase and tyrosine aminotransferase already has been reported for bovine growth hormone [14] and glucose [15]. To this list adrenaline is now added (Table 3). Adrenaline (0.04 to 0.15 mg/rat) was a potent inhibitor of induction of both the enzymes. The effect of growth hormone is now confirmed with the hormone obtained from the rat. The effect of glucose was obtained on pyrrolase at a concentration of 200 mg/rat, and not at 100 mg/rat, and a large dose of 2 g/rat led to flooding of the intraperitoneal cavity with fluid without, however, increasing the extent of inhibition. The results shown in Table 3 indicate non-selective inhibition of the two enzymes by adrenaline and rat growth hormone.

Effect of dihydroxyphenylserine. Administration of doses of 0.5, 1.0 and 5.0 mg/rat of DL-threo-3,4-dihydroxyphenylserine (DOPS) together with pargyline had no effect on the induction process of pyrrolase (results not shown). Administration of DOPS is known to result in elevated intracellular concentrations of noradrenaline due to the predominant localization of L-aromatic amino acid decarboxylase in the cytosolic compartment [16], which converts this compound to noradrenaline. These results further support the conclusion that noradrenaline exerts its inhibitory action on the cell membrane and not directly in the cytosol.

Effect of adrenergic agonists. The role of α-adrenergic receptors in the inhibition by noradrenaline was indicated by experiments on the reversal of the inhibition after treatment with α-adrenergic blocking agents but not with β-blockers [9]. The implication of membrane receptors in the inhibitory action of noradrenaline was explored further using adrenergic agonists which are analogues of the hormone capable of activating the specific receptors with greater efficacy. The use of agonists is particularly suitable in experiments *in vivo* where high concentrations of noradrenaline cannot be used because of their lethality. If the administered noradrenaline were not saturating the α-receptors, its effect would be enhanced by simultaneous treatment with another α-agonist. Similarly, even a β-agonist should enhance the action by displacement of noradrenaline in favor of α-receptors.

Table 4. Effect of adrenergic agonists on the inhibition by noradrenaline of induction by cortisol of hepatic tryptophan pyrrolase*

Treatment	Enzyme activity (nmoles/hr/mg protein)	
	− Noradrenaline	+ Noradrenaline
None	144 ± 21	72 ± 8
Phenylephrine	138 ± 15	40 ± 4†
Isoproterenol	123 ± 12	52 ± 2†

* All six groups of rats were treated with cortisol (10 mg/rat). Three of the groups were treated with noradrenaline (0.2 mg/rat) along with pargyline (5 mg/rat). Where specified, the adrenergic agonists, phenylephrine (0.5 mg/rat) or isoproterenol (4 mg/rat), were injected intraperitoneally 10 min before others. All animals were killed 3 hr after cortisol treatment. The values are expressed as mean ± S. E. M.

† Potentiation of noradrenaline inhibition by the agonists was significant, $P < 0.01$; the changes on treatment with agonists alone are not significant.

The results in Table 4 show that treatment of animals with phenylephrine (0.5 mg/rat), an α-agonist, together with pargyline, produced no inhibition of induction of pyrrolase and enhanced the inhibition obtained with noradrenaline. As expected, isoproterenol, a β-agonist, at a high concentration of 4 mg/rat showed no effect by itself and a small but definite enhancement of inhibition in the presence of noradrenaline. In all these experiments, tyrosine aminotransferase remained unaltered (results not shown).

Effect of catecholamines and rat growth hormone on stimulation of RNA synthesis. One of the striking effects, besides inducing the enzymes, of cortisol treat-

ment is the stimulation of incorporation of [^{32}P]-orthophosphate into RNA in the liver [17, 12]. In the last set of experiments, treatment with noradrenaline, adrenaline and rat growth hormone was given to see whether any differential effect on this stimulation of RNA synthesis would be found. Treatment with cortisol significantly stimulated incorporation of [^{32}P]-orthophosphate into RNA but treatment with the above three compounds, which inhibit the induction of the enzyme, showed no inhibition of RNA synthesis, in contrast to their effects on enzyme induction (Table 5). The inhibitory action of these compounds, therefore, is not via the gross stimulatory effect of RNA synthesis by cortisol. In view of the complexity of the process of stimulation of gene transcription by cortisol [18], it is premature to draw conclusions from this experiment on the lack of effect of noradrenaline at this stage of protein synthesis.

DISCUSSION

The most interesting finding in these studies is the specificity of noradrenaline for the selective inhibition of induction of tryptophan pyrrolase by cortisol. The availability of cortisol and its action on RNA synthesis are not the likely targets of this selective inhibition since under the experimental conditions tyrosine aminotransferase and incorporation of [^{32}P]-orthophosphate into RNA remain unaffected. The experiments with a variety of agents, albeit negative, give important clues, especially in eliminating involvement of changes in blood flow, glucose, several hormones, ATP, cyclic AMP, microtubules and RNA synthesis. The selective effect of noradrenaline, however, must be directed specifically against the particular protein, tryptophan pyrrolase, at one of the steps of its synthesis, yet to be identified.

The investigations of Exton *et al.* [8, 19] with adrenaline and that from this laboratory with noradrenaline implicating α-receptors in the enzyme induction process do not seem to support the possibility of direct mediation of cyclic nucleotides as the "second messengers". The participation of α-adrenergic receptors in a number of physiological and biochemical actions is being increasingly recognized.

Noradrenaline exhibits multiple effects on a variety of metabolic systems. Noradrenaline shows three different effects on the hepatic enzyme systems. First, noradrenaline inhibits the cortisol-mediated induction of cytosolic tryptophan pyrrolase acting via the α-receptor [9]; second, noradrenaline stimulates the mitochondrial succinate dehydrogenase, presumably via the β-receptor [20]; and third, noradrenaline, either given exogenously or generated within the cytosol from 3,4-dihydroxyphenylserine, increases the activity of microsomal 3-hydroxy-3-methylglutaryl (HMG) CoA reductase which is unaffected by the α- or the β-blocking agents [21]. A single hormone may thus have a variety of responses through different mechanisms in the same cell, and further work is needed to elucidate each of these mechanisms.

Table 5. Effect of noradrenaline, adrenaline and rat growth hormone on cortisol-stimulated incorporation of [^{32}P]-orthophosphate into RNA*

Treatment	[^{32}P]-orthophosphate incorporation (cpm/100 μg RNA)	
	Cortisol	Cortisol + compound
Noradrenaline	13,250 ± 930	12,950 ± 1810
Adrenaline	9,660 ± 900	10,800 ± 520
Rat growth hormone	15,800 + 1900	19,780 ± 3660

* Groups of rats were given, intraperitoneally, noradrenaline (0.2 mg/rat), adrenaline (80 μg/rat) or rat growth hormone (0.6 mg/rat). Those receiving noradrenaline and adrenaline were also given pargyline (5 mg/rat). All the animals were then given cortisol (10 mg/rat) and [^{32}P]-orthophosphate (0.5 mCi/rat) in quick succession. An absorbance of 2.0 was taken to correspond to 100 μg of RNA. The values are expressed as mean ± S. E. M. The changes obtained on cortisol treatment were significant at $P < 0.01$ when compared to the control group treated with saline (4910 ± 330 cpm/100 μg of RNA) but not to the changes obtained by the treatment with the compounds.

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