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Selective Induction of Tyrosine Hydroxylase and Dopamine β-Hydroxylase in Sympathetic Ganglia in Organ Culture: Role of Glucocorticoids as Modulators

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SUMMARY

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In organ cultures of rat sympathetic ganglia dexamethasone enhanced the synthesis of tyrosine hydroxylase and dopamine β-hydroxylase in a strictly dose-dependent manner. The maximal effect (tyrosine hydroxylase, +64%; dopamine β-hydroxylase, +47%) was achieved at 0.1 μM, and at 100 μM control levels were approached again. In contrast, the smaller effect on dopa decarboxylase (+24%) was virtually the same over the concentration range from 10 nM to 1 mM. For all the enzymes studied a 4-hr exposure of the ganglia to glucocorticoids was sufficient to produce a maximal effect after 48 hr. The glucocorticoid-mediated induction of tyrosine hydroxylase and dopamine β-hydroxylase was dependent on the presence of intact preganglionic cholinergic nerves. In decentralized ganglia the increase in tyrosine hydroxylase was reduced to +22%, and that of dopamine β-hydroxylase, to +4%. The increase in dopa decarboxylase remained unchanged. The addition of the ganglionic blocking agent chlorisondamine (10 μM) had the same effect as decentralization. In organ cultures originating from adrenalectomized animals carbamylcholine (100 μM) still produced a marked increase in tyrosine hydroxylase and dopamine β-hydroxylase. It is concluded that glucocorticoids exert a dual effect on sympathetic ganglia: (a) a strictly concentration-dependent induction of tyrosine hydroxylase and dopamine β-hydroxylase, which depends on the presence of intact preganglionic cholinergic nerves, and (b) a nonspecific general effect on protein synthesis, which is not dependent on intact preganglionic nerves and which is reflected by a small increase of all enzymes studied. Since specific induction of tyrosine hydroxylase and dopamine β-hydroxylase by carbamylcholine is still possible in ganglia from adrenalectomized animals, the synthesis of these two enzymes seems to be regulated primarily by the activity of the preganglionic nerves, with glucocorticoids playing a modulatory role.

INTRODUCTION

It is now well established and generally accepted that enhanced activity of the preganglionic cholinergic nerves leads to a selective increase in the synthesis of tyrosine hydroxylase [tyrosine 3-monooxygenase, EC 1.14.16.2; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating)] and dopamine β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (hydroxylating), EC

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1.14.17.1] in the terminal adrenergic neurons and adrenal chromaffin cells (1–3). Other enzymes involved in the synthesis or metabolic degradation of the adrenergic transmitter norepinephrine, such as dopa decarboxylase (3,4-dihydroxy-L-phenylalanine carboxylase, EC 4.1.1.28) and monoamine oxidase, remain unchanged (4–7). This neuronally mediated enzyme induction can be abolished not only by transection of the preganglionic cholinergic nerves (5, 8–10) but also by nicotinic blocking agents (11, 12). This suggests that the first messenger in this trans-synaptic enzyme induction is acetylcholine. This assumption is further supported by the observation that acetylcholine (13, 14) and carbamylcholine (15) can mimic the effect of increased preganglionic activity in decentralized adrenals.

Moreover, in recent experiments it has been shown that pulses of acetylcholine or carbamylcholine can trigger the selective induction of tyrosine hydroxylase and dopamine β-hydroxylase in sympathetic ganglia kept in organ culture (16).

At a very early stage in the investigation of the mechanism of tyrosine hydroxylase induction in the adrenal medulla it was shown that induction of this enzyme could be achieved in hypophysectomized rats (9). This observation suggests that the pituitary-adrenal axis is not primarily involved in the regulation of the synthesis of tyrosine hydroxylase, in contrast to that of phenylethanolamine *N*-methyltransferase, which is predominantly regulated by glucocorticoids (17, 18). However, in recent experiments it has been shown that the inducibility of tyrosine hydroxylase in superior cervical ganglia is subject to a diurnal rhythm and that this rhythm is determined by the diurnal variation in the production of glucocorticoids (19).

Previous experiments showed that it is possible to follow the whole process of trans-synaptic induction in organ cultures of sympathetic ganglia and that the time course is very similar to that *in vivo* (16). This system *in vitro* seemed to provide favorable conditions for studying in more detail the role of glucocorticoids in trans-synaptic induction.

MATERIALS AND METHODS

Experimental procedures. Female Sprague-Dawley rats (120–150 g) were killed by a blow on the head. The superior cervical ganglia were rapidly removed under sterile conditions, desheathed under a dissecting microscope, and placed on lens tissue lying on metal grids in sterile plastic culture dishes containing 1 ml of incubation medium. The details of the dissecting and incubation procedure have been described previously (16). Particular care was taken not to injure postganglionic fibers and to make sure that the postganglionic fiber bundles were at least 5–7.5 mm long. BGJ_b medium (modified according to Fitton-Jackson as indicated in the Gibco catalogue) supplemented with 10% fetal calf serum and freshly prepared glutamine, which was added to a concentration of 2 mM, was used for all experiments. Incubation was carried out in an atmosphere of 87% O_2–13% CO_2 at 36° in a humidified incubator for up to 72 hr. The culture medium was replaced after 48 hr. The pH of the medium was maintained at 7.4. Compounds to be tested were each added in a volume of 10 μl. Adrenalectomy was performed 14 days, and decentralization of the right superior cervical ganglia 7 days, prior to the beginning of the organ culture experiments.

Enzyme preparation. After incubation ganglia were rinsed thoroughly in Dulbecco's phosphate-buffered NaCl. One ganglion was homogenized in 0.5 ml of ice-cold 0.005 M Tris buffer (ph 7.4) containing 0.1% Triton X-100. The homogenates were centrifuged at 27,000 × *g* for 20 min. Control ganglia *in vivo* were dissected, desheathed, and homogenized immediately after removal from the animal.

Enzyme analyses. Radioactivity was determined in toluene containing 6 g of (2,4''-*tert*-butylphenyl)-5-(4''-biphenyl-1,3,4-oxidazole) and 300 ml of Triton X-100 per 1000 ml, except for dopamine β-hydroxylase, where the Triton X-100 was omitted. Protein concentations were determined according to Lowry *et al.* (20).

Tyrosine hydroxylase. The activity of this enzyme was assayed according to Levitt *et al.* (21) with modifications described

in detail by Mueller *et al.* (22), using 100 μl of enzyme preparation and with concentrations of 15 μM L-tyrosine and 720 μM 6,7-dimethyl-5,6,7,8-tetrahydropteridine HCl. The assay is based on the liberation of half the total tritium from the substrate 3',5'-ditritiotyrosine upon hydroxylation.

Dopamine β-hydroxylase. The activity of this enzyme was measured in 75-μl samples of the enzyme preparation according to Molinoff *et al.* (23) with modifications described by Oesch *et al.* (24). The substrate (phenylethylamine) concentration was 1 mM, and an optimal concentration of $CuSO_4$ (10 μM) was used in order to inactivate endogenous inhibitors. After homogenization in 0.005 M Tris buffer (pH 7.4) containing 0.1% Triton X-100, more than 90% of the enzymatic activity was present in the 27,000 × *g* supernatant.

Dopa decarboxylase. The enzyme activity was determined in 50-μl samples of the enzyme preparation according to Håkanson and Owman (25) with modifications described in detail by Oesch *et al.* (24). The assay is based on the conversion of radioactive dopa to dopamine in the presence of a monoamine oxidase inhibitor. The reaction product, [^{3}H]dopamine, was separated from unreacted substrate, [^{3}H]dopa, on Dowex 50-X4 (Na^+). The concentration of L-[^{3}H]dopa in the assay was 1 mM; pyridoxal phosphate, 0.24 mM; and tranylcypromine, 1.2 mM.

Monoamine oxidase. The activity was determined in 25 μl of the whole homogenate by the method of Wurtman and Axelrod (26).

Statistics. Student's *t*-test was used for determination of the significance of differences between means (27). The measure of variation in this study is the standard error of the mean.

Materials. L-[*ring*-3,5-^{3}H]Tyrosine (specific radioactivity, more than 30,000 mCi/mmole), L-[*side chain*-2,3-^{3}H]dopa (specific radioactivity, more than 1000 mCi/mmole), and *S*-adenosyl-L-[*methyl*-^{3}H]-methionine (specific radioactivity, 5000 mCi/mmole) were purchased from the Radiochemical Centre, Amersham. [*side chain*-2-^{14}C]Tryptamine bisuccinate (specific radioactivity, more than 85 mCi/mmole) was obtained from New England Nuclear Corporation; 6,7-dimethyl-5,6,7,8-tetrahydropteridine HCl, B grade, from Calbiochem; dexamethasone, cycloheximide, and carbamylcholine chloride, from Sigma Chemical Company; eserine, from Fluka, Buchs, SG, Switzerland; and chlorisondamine and (2,4''-*tert*-butylphenyl)-5-(4''-biphenyl-1,3,4-oxidazole), from Ciba-Geigy, Basel. Tissue culture media and other solutions, including modified BGJ_b medium, fetal calf serum, Dulbecco's phosphate-buffered NaCl, and glutamine (200 mM), were obtained as sterile solutions from Gibco. Organ culture dishes and grids were obtained from Falcon Plastics.

RESULTS

Time course of tyrosine hydroxylase, dopamine β-hydroxylase, and dopa decarboxylase activities in sympathetic ganglia kept in organ culture. The activities of these three enzymes were determined 18, 24, 48, and 72 hr after the superior cervical ganglia had been placed in culture. Within the first 48 hr none of the activities differed significantly ($p > 0.05$) from zero-time controls. However, after 72 hr all three enzymes had fallen below their initial levels. Tyrosine hydroxylase was reduced to 86%, dopamine β-hydroxylase, to 63%, and dopa decarboxylase, to 87%.

Light and electron microscopic studies performed on ganglia kept in organ culture up to 48 hr in the presence and absence of dexamethasone confirmed the survival of the neuronal cells, as indicated by the sustained enzyme levels over this period.

Concentration dependence of dexamethasone effect. The induction of both tyrosine hydroxylase and dopamine β-hydroxylase showed a strict dependence on the concentration of dexamethasone used. For both enzymes the maximal response was achieved at 0.1 μM (Fig. 1). Interestingly, at higher concentrations the maximal response was no longer maintained but gradually decreased, approaching control levels at 0.1–1 mM. In contrast, dopa decarboxylase activity did not reveal such a strict concentration dependence. As with tyrosine hydroxylase and dopamine β-hy-

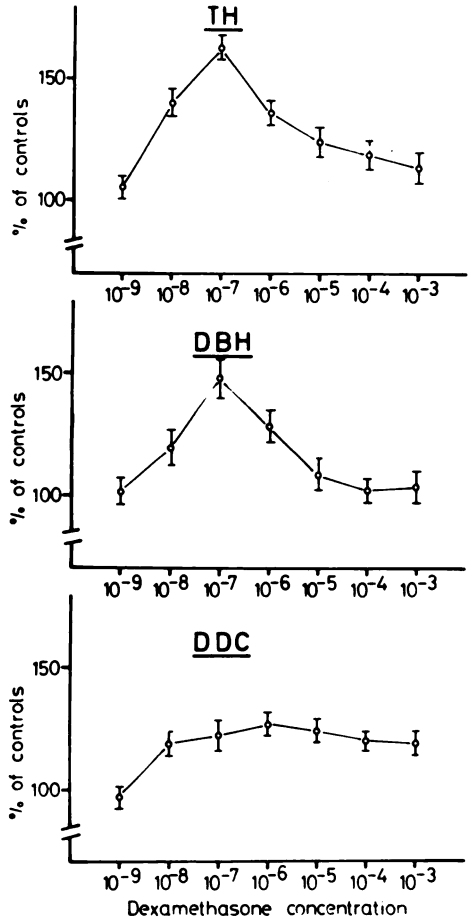

FIG. 1. *Concentration dependence of dexamethasone effect on enzyme levels in rat superior cervical ganglia in organ culture*

Superior cervical ganglia prepared for organ culture as described in detail under MATERIALS AND METHODS were exposed for 24 or 48 hr to varying concentrations of dexamethasone. After this time the ganglia were washed in phosphate-buffered NaCl and homogenized, and the activities of dopamine β-hydroxylase (DBH) (24 hr), tyrosine hydroxylase (TH) (48 hr), and dopa decarboxylase (DDC) (48 hr) were compared with those of ganglia kept in normal media for the duration of the experiment. The values represent the means ± standard errors of eight ganglia. They are expressed as a percentage of culture controls. The enzyme activity amounted to 1.61 ± 0.11 nmoles of dopa per hour per milligram of protein for tyrosine hydroxylase, to 99 ± 7 nmoles of phenylethanolamine per hour per milligram of protein for dopamine β-hydroxylase, and to 582 ± 28 nmoles of dopamine per hour per milligram of protein for dopa decarboxylase.

droxylase, the threshold concentration was 10 nM, but there was no further statistically significant ($p > 0.05$) change up to 1 mM. The maximal increase in tyrosine hydroxylase activity achieved by dexamethasone amounted to 63%; that of dopamine β-hydroxylase, to 46%; and that of dopa decarboxylase, to 24%. Similar results were obtained with corticosterone, the major physiological glucocorticosteroid in the rat. The maximal effect was obtained at a concentration of 3 μM. However, preference was given to the use of dexamethasone on account of its good water solubility.

Relationship between period of exposure to dexamethasone and subsequent increase in tyrosine hydroxylase and dopa decarboxylase. In order to determine the minimal time of exposure necessary for dexamethasone to initiate its maximal effect, we studied the response of ganglia to 0.1 μM dexamethasone for 1–48 hr. As shown in Fig. 2, the maximal increase in both enzyme activities after 48 hr was achieved after exposure for 4 hr. For tyrosine hydroxylase a statistically significant ($p < 0.025$) increase could be initiated by exposing superior cervical ganglia to 0.1 μM dexamethasone for a period as short as 1 hr.

Time course of enzyme changes in response to 0.1 μM dexamethasone exposure for 4 hr. The time course of the activities of tyrosine hydroxylase, dopamine β-hydroxylase, and dopa decarboxylase in superior cervical ganlia kept in organ culture after exposure to dexamethasone for 4 hr is demonstrated in Fig. 3. The maximal increase in dopamine β-hydroxylase (+47%) was reached at 24 hr. The maximal level of tyrosine hydroxylase (+64%) was not attained before 48 hr. The maximal increase in dopa decarboxylase amounted to +23% after 48 hr and returned to control levels after 72 hr. The values for monoamine oxidase were determined at 48 and 72 hr, and the increases amounted to +22% and +26%, respectively.

Effect of decentralization on dexamethasone-induced enzyme changes in cultured superior cervical ganglia. Experiments *in*

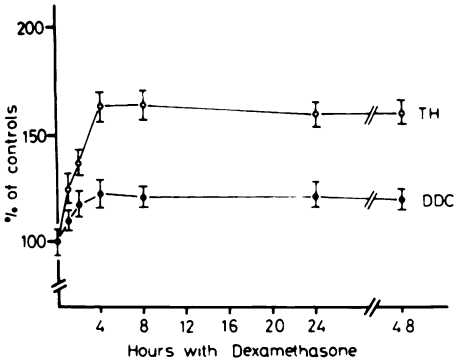

FIG. 2. *Relationship between duration of exposure to dexamethasone and subsequent changes in tyrosine hydroxylase (TH) and dopa decarboxylase (DDC) activity*

Superior cervical ganglia were exposed for 1-48 hr to 0.1 μM dexamethasone. In the experiments in which the ganglia were not exposed to dexamethasone for the whole duration of the incubation period (48 hr) the ganglia were placed in culture dishes which contained normal culture medium for the rest of the experiment. The values are expressed as a percentage of culture controls. Activities amounted to 1.84 ± 0.12 nmoles of dopa per hour per milligram of protein for tyrosine hydroxylase and to 572 ± 35 nmoles of dopamine per hour per milligram of protein for dopa decarboxylase. The values represent the means ± standard errors of eight ganglia.

vivo had shown that the increase in tyrosine hydroxylase activity in superior cervical ganglia elicited by large doses of dexamethasone depend on intact preganglionic cholinergic innervation (28). In order to establish whether the experiments in organ culture are representative for the situation *in vivo*, we studied the effect of dexamethasone on ganglia in organ culture which had been decentralized *in vivo* 7 days previously. As illustrated in Fig. 4, the effect of 0.1 μM dexamethasone on these two enzymes was markedly reduced by previous decentralization. The tyrosine hydroxylase increase was reduced from 64% to 22%, and that of dopamine β-hydroxylase, from 43% to nil. In contrast, the increase in dopa decarboxylase remained unchanged in decentralized ganglia. The nicotonic blocking agent chlorisondamine had an effect very similar to decentralization (Table 1).

Effect of cycloheximide on changes in enzyme activity produced by dexamethasone. In order to obtain information as to whether the effect of dexamethasone on tyrosine hydroxylase, dopamine β-hydroxylase, and dopa decarboxylase depends on intact protein synthesis, we studied the effect of cycloheximide. The concentration of cycloheximide used, 20 μM, reduced the total protein synthesis in organ cultures of sympathetic ganglia by 96% (as determined by the incorporation of [^{3}H]leucine into trichloracetic acid-precipitable protein) (29) and also abolished the effect of dexamethasone on all three enzymes (Table 2). Thus it seems that the increased activity of these enzymes results from an increased synthesis of new enzyme protein. This interpretation is further supported by recent experiments which have shown that the increase in dopamine β-

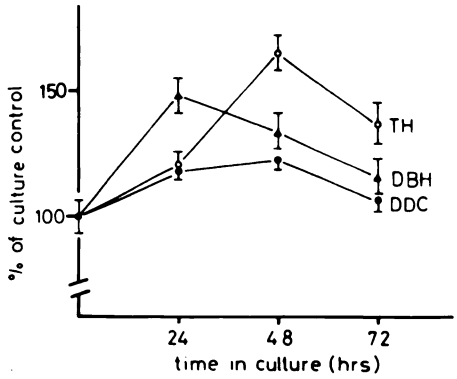

FIG. 3. *Time course of enzyme changes after exposure to 0.1 μM dexamethasone*

Superior cervical ganglia were exposed for 4 hr to 0.1 μM dexamethasone. Thereafter they were kept in culture for 20, 44, and 68 hr in normal media. At the end of a 24-, 48-, or 72-hr incubation period the ganglia were homogenized and their dopamine β-hydroxylase (DBH), tyrosine hydroxylase (TH), and dopa decarboxylase (DDC) activities were compared with the enzyme activities of ganglia kept in normal culture media for the whole incubation period. The values are expressed as a percentage of culture controls and represent the means ± standard errors of six or seven ganglia. The activity of uncultured controls amounted to 1.98 ± 0.11 nmoles of dopa per hour per milligram of protein for tyrosine hydroxylase, to 112 ± 9 nmoles of phenylethanolamine per hour per milligram of protein for dopamine β-hydroxylase, and to 584 ± 30 nmoles of dopamine per hour per milligram of protein for dopa decarboxylase.

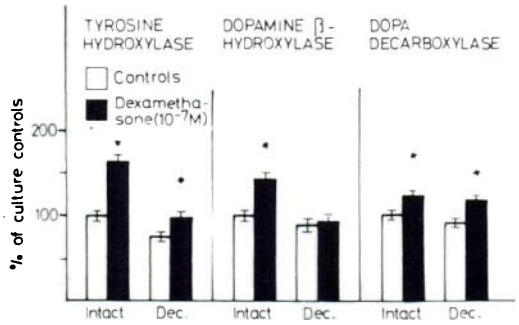

FIG. 4. *Effect of decentralization on dexamethasone-mediated enzyme induction*

Superior cervical ganglia were decentralized 7 days prior to the beginning of the organ culture experiments. Decentralized (Dec.) and intact ganglia were incubated for 4 hr in 0.1 μM dexamethasone, then washed in phosphate-buffered NaCl and placed in normal medium for a further 20 or 44 hr. After a total incubation time of 24 or 48 hr the ganglia were homogenized and the activities of dopamine β-hydroxylase (24 hr), tyrosine hydroxylase (48 hr), and dopa decarboxylase (48 hr) were compared with ganglia kept in normal media during the whole duration of the experiment. The values represent the means ± standard errors of seven or eight ganglia and are expressed as a percentage of intact culture controls. The enzyme activity amounted to 1.70 ± 0.14 nmoles of dopa per hour per milligram of protein for tyrosine hydroxylase, to 102 ± 6 nmoles of phenylethanolamine per hour per milligram of protein for dopamine β-hydroxylase, and to 566 ± 31 nmoles of dopamine per hour per milligram of protein for dopa decarboxylase.

hydroxylase and dopa decarboxylase activities produced by dexamethasone in organ cultures of sympathetic ganglia parallels an increased incorporation of [^{3}H]leucine into the protein fractions which are specifically precipitated by monospecific antibodies to these two enzymes.[1]

Effect of carbamylcholine on tyrosine hydroxylase activity in organ cultures of superior cervical ganglia originating from adrenalectomized rats. In order to determine whether glucocorticoids are an absolute prerequisite for trans-synaptic enzyme induction or whether they act as modulators of the acetylcholine-mediated process, we studied the effect of adrenalectomy on enzyme induction by carbamylcholine. This approach was chosen after preliminary experiments had shown that adrenalectomized rats did not survive any of the procedures suitable for producing increased preganglionic activity, such as cold stress or treatment with reserpine.

The results presented in Table 3 show that adrenalectomy performed 2 weeks before the ganglia were brought into culture had no statistically significant ($p > 0.05$) effect on the basal levels of tyrosine hydroxylase activity. Although the response to 100 μM carbamylcholine was smaller in ganglia originating from adrenalectomized rats than from intact ones, the increase was statistically significant ($p < 0.025$), indicating that trans-synaptic tyrosine hydroxylase induction is possible without glucocorticoids but that the induction is enhanced by adequate concentrations of these steroids.

DISCUSSION

It was the aim of the present study to establish the role of neuronal and hormonal factors in the process of trans-synaptic enzyme induction in sympathetic ganglia kept in organ culture.

In a series of previous studies it was shown that in sympathetic ganglia *in vivo*

TABLE 1

Effect of chlorisondamine on dexamethasone-mediated tyrosine hydroxylase increase in cultured rat sympathetic ganglia

Rat superior cervical ganglia were incubated for 4 hr with dexamethasone, chlorisondamine, or a combination of the two drugs. The ganglia were then transferred to normal media for a further 44 hr. Values represent the means ± standard errors of six ganglia.

Treatment	Tyrosine hydroxylase activity
	nmoles dopa/hr/mg protein
Controls	1.93 ± 0.14
Chlorisondamine (10 μM)[a]	2.18 ± 0.21
Dexamethasone (0.1 μM)	3.01 ± 0.17[b]
Chlorisondamine[a] + dexamethasone	2.34 ± 0.18

[a] The animals were injected with 5 mg/kg of chlorisondamine intraperitoneally 30 min before they were killed, and the ganglia were transferred to culture.

[b] $p < 0.025$ compared with controls.

[1] U. Otten and C. Gagnon, unpublished observations.

TABLE 2

Effect of cycloheximide on dexamethasone-mediated enzyme changes

Superior cervical ganglia from adult female Sprague-Dawley rats were maintained in organ culture for 48 hr. They were incubated in normal culture medium, in medium containing 0.1 μM dexamethasone or 20 μmoles of cycloheximide, or in medium containing both dexamethasone and cycloheximide. Cycloheximide was present during the entire 48-hr incubation period, and dexamethasone, for 4 hr only. Values are the means ± standard errors of eight ganglia.

Treatment	Tyrosine hydroxylase	Dopamine β-hydroxylase	Dopa decarboxylase
		nmoles product/hr/mg protein	
Culture controls	1.85 ± 0.11	86 ± 6	498 ± 20
Dexamethasone	3.26 ± 0.16[a]	114 ± 7[a]	589 ± 24[a]
Cycloheximide	1.67 ± 0.12	68 ± 5[b]	487 ± 21
Dexamethasone + cycloheximide	2.0 ± 0.12	64 ± 4[a]	464 ± 26

[a] $p < 0.01$ compared with culture controls.
[b] $p < 0.025$ compared with culture controls.

TABLE 3

Effect of previous adrenalectomy on carbamylcholine-mediated tyrosine hydroxylase induction in organ cultures of sympathetic ganglia

Superior cervical ganglia of control and adrenalectomized rats were decentralized 7 days prior to the beginning of the organ culture experiments. Decentralized ganglia from intact and adrenalectomized animals were exposed for 4 hr to 100 μM carbamylcholine, then kept in normal media for 44 hr. At the end of the incubation period the ganglia were homogenized and the activity of tyrosine hydroxylase was compared with that of ganglia kept in organ culture without carbamylcholine. The values represent the means ± standard errors of 8–10 ganglia.

Treatment	Tyrosine hydroxylase	
	Decentralized	Decentralized + adrenalectomized
	nmoles dopa/hr/mg protein	
None	1.79 ± 0.09	1.68 ± 0.06
Carbamylcholine (100 μM)	2.36 ± 0.11[a]	2.02 ± 0.09[a]

[a] $p < 0.025$.

and *in vitro* glucocorticoids can influence the level of enzymes involved in the synthesis and metabolic degradation of the adrenergic transmitter norepinephrine (19, 28, 30–32). In recent experiments Hanbauer *et al.* (28) have shown that high doses of dexamethasone elicited the induction of tyrosine hydroxylase in adult rats. Since the induction was dependent on intact preganglionic cholinergic fibers, it could not be decided whether dexamethasone was the factor of primary importance and the presence of intact preganglionic cholinergic nerves represented merely a permissive factor, or whether the increased preganglionic activity was of predominant importance and dexamethasone acted as a modulator. The latter assumption was suggested by our recent observation that trans-synaptic induction of tyrosine hydroxylase in sympathetic ganglia exhibits a diurnal rhythm which is positively correlated with the diurnal rhythm of the synthesis of adrenal glucocorticoids (19).

The present experiments strongly support the concept of a modulatory role of glucocorticoids in trans-synaptic induction. They have shown that the specific induction of tyrosine hydroxylase and dopamine β-hydroxylase elicited by dexamethasone in organ cultures of sympathetic ganglia depends on the presence of preganglionic cholinergic nerves. In ganglia which had been decentralized 7 days prior to the beginning of the organ culture experiment, the specific induction of these two enzymes could not be elicited by dexamethasone. Accordingly, in the presence of intact preganglionic cholinergic nerves, the specific effect of dexamethasone could be abolished by chlorisondamine, a nicotinic ganglionic blocking agent.

In contrast, in ganglia originating from animals which had been adrenalectomized 2 weeks prior to the beginning of the organ culture experiments, a specific induction

of tyrosine hydroxylase could still be elicited with carbamylcholine without the addition of glucocorticoids to the culture medium. These findings strongly support the concept that the primary factor in this specific enzyme induction is the increased activity of the preganglionic fibers and that glucocorticoids play a true modulatory role.

It seems that the regulatory role of glucocorticoids is not confined to the adrenergic neurons but is also present in the adrenal medullary cells. This can be deduced from the observation that tyrosine hydroxylase induction by short-term cold stress is subjected to a circadian rhythm not only in the sympathetic ganglia but also in the adrenal medulla (19). However, in contrast to the ganglia, optimal induction in the medulla occurs during the period of lowest glucocorticoid production. Since the adrenal medulla is exposed to very high steroid concentrations—one to two orders of magnitude higher in the blood of the adrenal medulla than in the general circulation—it could be assumed that the optimal glucocorticoid concentrations for trans-synaptic induction in the adrenal medulla are reached during the time of lowest steroid production, whereas for induction in the adrenergic neurons the time of maximal steroid production is optimal. This assumption is further supported by the observation that in newborn animals, in which the diurnal rhythm of glucocorticoid production is not yet developed (33) and glucocorticoid production is low (34), short-term cold stress can initiate tyrosine hydroxylase induction in the adrenal medulla at any time of the day. However, tyrosine hydroxylase induction in sympathetic ganglia by short-term cold exposure is not possible at all unless the newborn animals have been treated with glucocorticoids. Thus these experiments performed *in vivo* strongly suggest that the modulatory role of glucocorticoids in trans-synaptic induction is concentration-dependent and that the highest concentrations reached in the adrenal medulla are inhibitory. This assumption is also supported by recent observations in the rat adrenal medulla in organ culture (35). In these experiments tyrosine hydroxylase induction was initiated by administration of reserpine *in vivo*. Four hours after the injection the animals were killed and the adrenal medullae were transferred to culture. The induction initiated *in vivo* by reserpine progressed in organ culture in a very similar way. If the organ cultures were supplied immediately with high concentrations of glucocorticoids, the increase in tyrosine hydroxylase could be blocked. However, if the cultures were supplied with the same doses of glucocorticoids 4 hr later, they had no influence on the process of tyrosine hydroxylase induction.

In the present experiments the concentration dependence of glucocorticoid action on sympathetic ganglia in organ culture was investigated in more detail. It was shown that the concentration of dexamethasone producing optimal induction of tyrosine hydroxylase and dopamine β-hydroxylase is rather specific, i.e., 0.1 μM. In contrast, the much smaller response of dopa decarboxylase was virtually the same over a concentration range from 10 nM to 1 mM. Moreover, the increase in dopa decarboxylase and monoamine oxidase activities was not dependent on the presence of intact preganglionic cholinergic nerves. Thus it can be concluded that the strictly concentration-dependent modulatory role of glucocorticoids is confined to tyrosine hydroxylase and dopamine β-hydroxylase, the two enzymes selectively involved in trans-synaptic induction. However, it can also be concluded that glucocorticoids have an additional effect which remains unchanged over a large concentration range, which is independent of the presence of cholinergic nerves, and which seems to be rather nonspecific, representing a general effect on protein synthesis.

In conclusion, glucocorticoids seem to have a dual effect: (a) a strictly concentration-dependent modulatory effect on the induction of tyrosine hydroxylase and dopamine β-hydroxylase, which is predominantly mediated by the activity of the preganglionic cholinergic nerves, and (b) a nonspecific effect, which is reflected by a small increase in dopa decarboxylase and monoamine oxidase, enzymes which are never augmented to a significant extent under experimental conditions which lead

to trans-synaptic induction of tyrosine hydroxylase and dopamine β-hydroxylase *in vivo*.

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