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EFFECT OF DEXAMETHASONE ON BIOPTERIN LEVELS AND TYROSINE HYDROXYLASE ACTIVITY IN PC-12 CELLS

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Abstract—Culture of PC-12 cells in 1 μM dexamethasone for 24, 48 or 72 hr did not alter significantly PC-12 cell total biopterin levels, although tyrosine hydroxylase activity in extracts of cell homogenates was increased 2- to 3-fold. Increasing the concentration of dexamethasone to 10 μM did not change biopterin levels or result in further increases in tyrosine hydroxylase activity measured after 72 hr. Culture of cells in dexamethasone markedly decreased the ratio of biopterin concentration to tyrosine hydroxylase activity. The molar ratio of tyrosine hydroxylase subunits to biopterin in control cells can be estimated to be approximately one. Therefore, following induction of the enzyme by dexamethasone, there appears to be an excess of enzyme molecules relative to cofactor molecules in these cells.

6*R*-(L-Erythro-1′,2′-dihydroxypropyl)-2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine (tetrahydrobiopterin; BH_4‡) is the pteridine cofactor for a number of monooxygenase enzymes, including tyrosine and tryptophan hydroxylases which catalyze the rate-limiting steps in catecholamine [1] and serotonin [2] syntheses respectively. The intracellular concentration of BH_4 is thought to be an important factor in the short-term regulation of tyrosine hydroxylase (tyrosine 3′-monooxygenase; EC 1.14.16.2) activity. Several lines of evidence suggest that it may be present at subsaturating concentrations for the enzyme [3, 4]. Phosphorylation of tyrosine hydroxylase, mediated by a cyclic AMP-dependent protein kinase, results in activation of the enzyme [5, 6]. Although there is some controversy over the kinetic expression of this activation [5, 7], the major kinetic changes seem to be a marked decrease in K_m for the pteridine cofactor [8] and an increase in K_i for dopamine [9]. Thus, a knowledge of the intracellular concentration of BH_4 relative to the K_m values of the phosphorylated and nonphosphorylated forms of the enzyme is critical to an understanding of its role in short-term regulation of tyrosine hydroxylase. An estimate of at least 100 μM BH_4 has been made for striatal dopaminergic neurons [10, 11], resulting in the hypothesis that the kinetic changes accompanying phosphorylation may convert a virtually inactive form of tyrosine hydroxylase to a fully active one [11, 12]. According to this hypothesis, BH_4 would be subsaturating only for the inactive, high K_m form of the enzyme. A somewhat lower estimate of BH_4 concentration (11 μM) in adrenal medullary chromaffin cells has led to the alternative proposal that BH_4 is subsaturating for both low and high K_m forms of adrenal tyrosine hydroxylase [13].

BH_4 levels may also be important in the long-term regulation of tyrosine hydroxylase activity. It is well established that tyrosine hydroxylase is induced in adrenal medulla and in peripheral and central catecholaminergic neurons in response to chronic and intense neurogenic stimulation and/or catecholamine depletion [14–16]. Increased levels of BH_4 have been reported recently to accompany tyrosine hydroxylase induction and increased tyrosine hydroxylation in the adrenal medullae of rats treated with insulin or reserpine [13]. Cultured bovine adrenal chromaffin cells in which tyrosine hydroxylase is induced by exposure of cells to tetrabenazine or 8-bromo-cyclic AMP also have elevated levels of BH_4 [17]. The elevation of BH_4 is thought to result from increased activity and induction of the enzyme GTP cyclohydrolase [18] which probably catalyzes the rate-limiting step in BH_4 biosynthesis [19]. It has been suggested, therefore, that the constant increased demand for BH_4 results in a long-term elevation of BH_4 and that tyrosine hydroxylase and GTP cyclohydrolase may undergo coordinate regulation [20].

The present study has examined the relationship of total biopterin to tyrosine hydroxylase levels in PC-12 cells, a clonal cell line derived from a rat pheochromocytoma [21]. PC-12 cells express many of the characteristics of differentiated noradrenergic neurons, including the capacity for synthesis, storage, uptake and release of catecholamines [21, 22]. Furthermore, PC-12 cell tyrosine hydroxylase resembles the enzyme from other tissues in many of its properties [23] and undergoes short-term regulation [24]. The present study has investigated total biopterin levels in PC-12 cells in which tyrosine hydroxylase has been induced by exposure of the cells to the synthetic glucocorticoid dexamethasone.

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‡ Abbreviations: BH_4, tetrahydrobiopterin; qBH_2, quinonoid dihydrobiopterin; BH_2, dihydrobiopterin; and HPLC, high performance liquid chromatography.

MATERIALS AND METHODS

Cell culture. PC-12 cells were supplied by Dr. Gordon Guroff (Laboratory of Developmental Neurobiology, NIH). The cells were maintained in 250-ml Costar flasks (growth area 75 cm^2) in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 5% horse serum. The horse serum was heat-inactivated at 60° for 45 min before use. The cells were grown in a humidified atmosphere in 10% CO_2 at 35° and subcultured every 7 days.

For experimental studies, cells (passage number 48–51) were subcultured in 50-ml Costar flasks (growth area 25 cm^2) at an approximate plating density of 10^6 cells/flask in 5 ml of medium. The cells were treated 24 hr after plating with medium containing 0.01% ethanol (vehicle control) or 1 μM dexamethasone in 0.01% ethanol. The dexamethasone medium was prepared from a 10 mM solution of dexamethasone in ethanol, made sterile by filtration through a 0.22 μm HA Millipore filter. The vehicle control medium was prepared from ethanol in a similar manner. Media were changed every 24 hr. Neither the vehicle control nor the dexamethasone medium affected the morphology of the cells viewed by light microscopy. Culture of cells in dexamethasone did not appear to alter cellular protein levels. At various times after initiation of treatment, the cells were detached by a sharp blow to the growth side of the flask, divided into two roughly equal aliquots, and harvested by centrifugation at 800 *g* for 4 min. The pellets were washed once with 5 ml of phosphate-buffered saline (0.9% NaCl, 10 mM sodium phosphate, pH 7.4) at room temperature and frozen immediately on dry ice for assay of biopterin and tyrosine hydroxylase activity.

Biopterin assay. Frozen cell pellets were thawed and immediately homogenized in 400 μl of ice-cold 0.1 M HCl. An aliquot of homogenate was frozen for protein assay. Duplicate aliquots of the homogenates were assayed for total biopterin by a modification of the method of Fukushima and Nixon [25]. Assay tubes contained 60 μl of homogenate and 2 ng D-erythroneopterin in 0.1 N HCl as an internal standard in a final volume of 140 μl. The final pH of the reaction mixture was less than 1. Oxidation was initiated by addition of 30 μl of acid iodine (0.5% iodine, 1% KI in 0.2 M trichloroacetic acid) and allowed to proceed for 1 hr at room temperature in subdued light.

Biopterin and neopterin in the oxidized samples were partially purified by ion-exchange chromatography by a modification of the method described in Ref. 25. Each sample was applied to a 0.6 × 1 cm column of Dowex 50W × 8 (hydrogen form, 200–400 mesh), the transferring pipette and tube were washed with 1 ml of water, and wash fluid was transferred to the column. Columns were then washed with 5 ml of water, and biopterin and neopterin were eluted with 6 ml of 0.25 M ammonium hydroxide. The ammonia eluates were applied directly onto separate 0.6 × 0.6 cm columns of Dowex 1 × 8 (chloride form, 200–400 mesh). The latter columns were washed with 2.5 ml of water, and biopterin and neopterin were eluted with 2 ml of 1 M HCl. The eluates were lyophilized, and the residues were resuspended in water and assayed for biopterin and neopterin by reverse phase high performance liquid chromatography (HPLC) with fluorescence detection (350/445 nm). Chromatography was performed on a Chromanetics 5 μm Spherisorb ODS column (250 × 4.5 mm) with a mobile phase of 10% methanol in water and a flow rate of 1.3 ml/min. Biopterin was quantitated by peak height ratio analysis from a standard curve of biopterin determined in the presence of PC-12 cell homogenate taken through the entire procedure. The final recoveries of biopterin and neopterin throughout the procedure were 60–70%. Preliminary experiments showed that reduced forms of biopterin were oxidized to biopterin [>90% conversion of BH_4 and dihydrobiopterin (BH_2)] and biopterin itself was stable to the oxidation, therefore resulting in a measurement of total biopterin.

In other experiments, separate aliquots of the same homogenates were subjected to either acid or basic iodine oxidation in order to determine which forms of biopterin were present in PC-12 cell extract [25]. Acid oxidation was carried out as described above and basic oxidation as described in Ref. 25. Preliminary experiments showed that >90% of BH_2 and <6% of BH_4 were converted to biopterin under the conditions of basic oxidation. Subtraction of the basic oxidation value from the acid oxidation value gives a measure of the amount of (BH_4 + qBH_2) present in the homogenate, whereas the basic oxidation value gives a measure of the amount of (BH_2 + B).

In another series of experiments, total biopterin in the media of control PC-12 cells was determined by acid iodine oxidation as described above.

Tyrosine hydroxylase activity. Frozen cell pellets (about 400–800 μg protein, depending on culture time) were thawed and immediately homogenized in 200 μl of ice-cold 50 mM potassium phosphate, pH 6.0, containing 0.1% Triton X-100. An aliquot of the homogenate was taken for protein assay and the remainder was centrifuged at 30,000 *g* for 15 min. The supernatant fraction, which contained >90% of the tyrosine hydroxylase activity in the homogenate, was used for the assay without further treatment. Preliminary experiments showed that passing the supernatant fraction through a small column of Sephadex G-25 (23 × 0.5 cm), which removed >90% of endogenous catecholamines as measured by HPLC with electrochemical detection (data not shown), did not alter the enzyme activity.

Tyrosine hydroxylase activity was measured by assaying the amount of tritiated water released during the conversion of L-[3,5-^{3}H]tyrosine to DOPA [8, 26]. Each assay tube contained 250 mM potassium phosphate, pH 6.24; 20 mM ascorbic acid; 0.1 mM ferrous ammonium sulfate; 5500 units of catalase; 0.1 mM L-tyrosine containing 100,000 cpm of L-[3,5-^{3}H]tyrosine; 1 mM BH_4; and 40 μl of tissue extract in a final volume of 100 μl. Blanks contained homogenizing medium instead of tissues. The pH of the final incubation mixture was 6.0. The reaction was started by the addition of 40 μl of tissue or homogenizing medium and incubation was carried out at 37° for either 14 or 4 min. The reaction was stopped by adding 400 μl of 5% trichloroacetic acid. Tritiated

water was isolated by passing each mixture through a small column consisting of a pasteur pipette plugged with glass wool and containing, from the bottom upwards, 2 cm of AG 50W × 4 resin, 200–400 mesh, H^+ form, a thin layer of activated charcoal and 1 cm of AG 1 × 2 resin, 200–400 mesh, acetate form. Effluents were collected directly into scintillation vials. The reaction tubes were washed twice with 700 μl of water and the washes also were collected. Radioactivity in the total eluate was determined in a liquid scintillation counter after addition of 15 ml of ACS to the vials. Tyrosine hydroxylase activity was calculated based on the assumption that only one of the two tritium atoms on tyrosine was released.

Tyrosine hydroxylase activity of PC-12 cell supernatant fractions was linear with protein up to at least 4 μg protein, the largest amount of protein assayed. Preliminary experiments showed, however, that activity was not linear with time, since there was always a lag of at least 1 min before linearity with time occurred. The reason for this consistent phenomenon, which was independent of protein concentration and which has also been observed for rat striatal tyrosine hydroxylase (Dr. Leonard Miller, personal communication), is not known. To overcome this, the amount of tritium released was measured at both 14 and 4 min, during which linear product formation occurred, and activity during the 10-min period was determined by subtraction. The ratio of cpm in the blanks to total added cpm ranged from 0.3 to 0.4%.

Protein assay. Protein in cell homogenates was assayed by the method of Bradford [27] using bovine gamma globulin as a standard.

Materials. L-[3,5-^{3}H]Tyrosine, sp. act. 52 Ci/mmole, was purchased from Amersham (Arlington Heights, IL) and purified before use by adsorption and elution from a small column of Dowex 50 X 4 resin, 200–400 mesh, H^+ form [26]. Dexamethasone was obtained from the Sigma Chemical Co. (St. Louis, MO). Culture media were purchased from GIBCO (Grand Island, NY) and sera from KC Biologicals (Lenexa, KS). (*R,S*)-BH_4·2HCl, biopterin and D-erythroneopterin were obtained from Dr. B. Schircks (Wetzikon, Switzerland).

Statistical analysis. The measure of variation used throughout this study is the standard error of the mean (S.E.). Student's *t*-test was used to determine the significance of the difference between means when a comparison of absolute values was made. When values were expressed as a percentage of another value and derived from several experiments, the weighted mean and appropriate confidence limits of the ratios of absolute values were determined.

RESULTS

Effect of dexamethasone in culture on PC-12 cell tyrosine hydroxylase activity. Culture of cells in medium containing 1 μM dexamethasone for 24, 48 and 72 hr resulted in a continuous increase in PC-12 tyrosine hydroxylase activity relative to ethanol controls when activity was measured in extracts of cell homogenates (Fig. 1). The increases were 1.5, 1.9 and 2.4 times control respectively (mean of three experiments). Tyrosine hydroxylase activity of

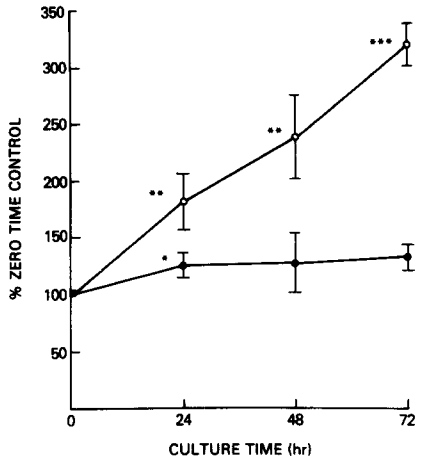

Fig. 1. Effect of 1 μM dexamethasone on tyrosine hydroxylase activity in PC-12 cells. The cells were plated at a density of 10^6 cells/25 cm^2 flask and treated 24 hr later (zero-time) with control medium containing 0.01% ethanol (●) or medium containing 1 μM dexamethasone in 0.01% ethanol (○). Media were changed daily. The cells were harvested and assayed for tyrosine hydroxylase activity as described in Materials and Methods. Each point shown is the mean ± S.E. of three separate experiments in which the tyrosine hydroxylase activity of four or five flasks of cells was assayed in duplicate. Enzyme activity in each experiment is expressed as a percentage of the zero-time value, which was 540 ± 100 nmoles L-DOPA/hr/mg protein. Key: (*) $P < 0.05$ vs zero-time value, and (**) $P < 0.01$ and (***) $P < 0.001$ vs ethanol control.

ethanol-treated control cells increased about 25–30% over zero-time activity during the 72-hr period, but the increase was statistically significant only at 24 hr. The inclusion of ethanol in the control medium had no effect on the tyrosine hydroxylase activity of PC-12 cells cultured for 48 hr (control without ethanol, 287.3 ± 30.7; ethanol control, 286.7 ± 11.6 nmoles L-DOPA/hr/mg protein; mean ± S.E., N = 5, single experiment). An increase in the medium concentration of dexamethasone to 10 μM did not result in any further enhancement of tyrosine hydroxylase activity measured after culture of PC-12 cells for 72 hr.

Effect of dexamethasone in culture on total biopterin in PC-12 cells. Total biopterin levels were measured in separate aliquots of the same cell suspensions used to assay tyrosine hydroxylase activity. A chromatogram of a PC-12 cell homogenate assayed for biopterin as described in Materials and Methods, but in the absence of the internal standard neopterin, is shown in Fig. 2b. When compared with pteridine standards (Fig. 2a), it is evident that PC-12 cell extracts contain a fluorescent compound with the same retention time as biopterin and that they do not contain detectable amounts of neopterin. Therefore, neopterin may be used as an internal standard in the quantitation of PC-12 cell biopterin. Figure 2c shows a chromatogram of a homogenate assayed for biopterin, where neopterin has been added as an internal standard. The peak with a retention time of 12 min in Fig. 2b and 2c is an artifact arising from the ammonium hydroxide elution of the Dowex 50

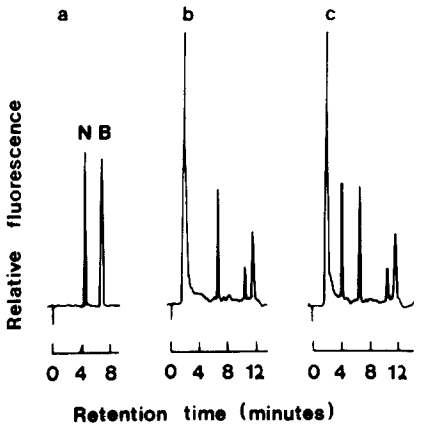

Fig. 2. Reverse phase HPLC analysis of PC-12 cell total biopterin by a modification of the method of Fukushima and Nixon [25]. See Materials and Methods for details of iodine oxidation and partial purification of biopterin and neopterin. Samples were chromatographed on a Chromanetics 5 μm Spherisorb ODS column (250 × 4.6 mm) with a mobile phase of 10% methanol in water at a flow rate of 1.3 ml/min. Biopterin and neopterin were detected by fluorescence detection (350/445 nm) on a Perkin–Elmer model 650-10 fluorescence detector. The scale of relative fluorescence on the vertical axis is identical for each chromatogram. (a) Chromatogram of a mixture of 0.2 ng each of neopterin (N) and biopterin (B). (b) Chromatogram of a PC-12 cell homogenate assayed for total biopterin as described in Materials and Methods, but in the absence of neopterin. (c) Chromatogram of a PC-12 cell homogenate assayed for total biopterin, in the presence of the internal standard neopterin.

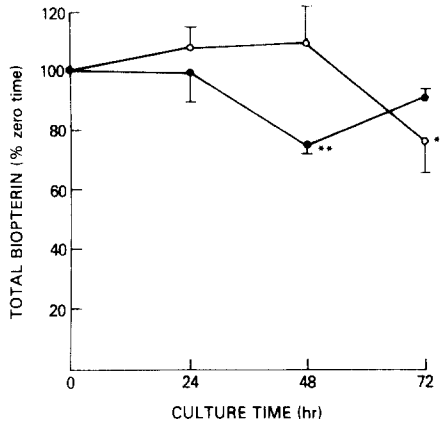

Fig. 3. Effect of 1 μM dexamethasone on total biopterin in PC-12 cells. The cells were plated at a density of 10^6 cells/25 cm^2 flask and treated 24 hr later (zero-time) with control medium containing 0.01% ethanol (●) or medium containing 1 μM dexamethasone in 0.01% ethanol (○). Media were changed daily. Cells were harvested and assayed for total biopterin as described in Materials and Methods. Each point shown is the mean ± S.E. of three separate experiments in which the biopterin levels of four or five flasks of cells were assayed in duplicate. Biopterin levels are expressed as percent of the zero-time value which was 17.9 ± 4.0 ng biopterin/mg protein. Key: (*) $P<0.05$ vs zero-time value, and (**) $P<0.01$ vs zero-time value and vs dexamethasone.

column and is also observed in blanks without PC-12 cell extract. The smaller peak with a retention time of about 11 min may be pterin, although the present study did not investigate this possibility.

PC-12 cell total biopterin in cells treated with 0.01% ethanol or 1 μM dexamethasone in 0.01% ethanol for 24, 48 or 72 hr is shown in Fig. 3. Values are expressed as a percentage of the zero-time value. Dexamethasone treatment appeared to result in little change in total biopterin although a decrease in biopterin levels at 48 hr of culture seen in the vehicle control was apparently prevented by this drug. Because of significant variation in baseline values of biopterin, the differences were not significant when absolute values were compared. If percent of control is used as a value, the dexamethasone cells had a larger amount of total biopterin at 48 hr of culture.

An increase in the medium concentration of dexamethasone to 10 μM did not alter biopterin levels relative to control measured after 72 hr of culture (ethanol control, 17.29 ± 2.04; 10 μM dexamethasone, 15.92 ± 1.34 ng biopterin/mg protein; mean ± S.E., N = 4; single experiment).

The effects of the vehicle, 0.01% ethanol, on biopterin levels in PC-12 cells were tested in a single experiment, where cells were cultured for 48 hr in the presence or absence of 0.01% ethanol. Inclusion of ethanol in the medium had no effect on biopterin levels in the cells (control without ethanol, 11.06 ± 1.25; ethanol control, 10.68 ± 0.53 ng biopterin/mg protein; mean ± S.E., N = 5).

When separate aliquots of control PC-12 cell homogenates were subjected to either acid or basic iodine oxidation and the amount of biopterin determined under both conditions, about 95% of the biopterin was found to occur in the homogenates as BH_4 and/or qBH_2 (60 μl cell homogenate: acid oxidation, 24.0 ng; basic oxidation, 1.5 ng biopterin).

Acid iodine oxidation of culture medium in which control PC-12 cells had been maintained for 5 days in 250-ml flasks showed that the medium contained only 22% of the total biopterin found in the cells (cells, 38.4 ng; medium, 8.4 ng biopterin).

Effect of dexamethasone in culture on the ratio of tyrosine hydroxylase activity to total biopterin in PC-12 cells. Dexamethasone significantly increased the ratio of tyrosine hydroxylase activity to total biopterin in PC-12 cells relative to ethanol controls (Table 1), with the largest effect observed at 72 hr (3-fold increase in ratio). This was due almost entirely to the dexamethasone-induced increase in tyrosine hydroxylase activity.

DISCUSSION

Prolonged neurogenic stimulation of adrenal glands and/or severe catecholamine depletion results in a marked elevation of adrenal tyrosine hydroxylase activity including an induction of new tyrosine hydroxylase protein [15]. Recent studies have reported that adrenal medullary BH_4 levels are also increased under these conditions [13], possibly due to induction of the enzyme GTP cyclohydrolase [18], which is thought to catalyze the rate-limiting step in BH_4 biosynthesis. In view of the importance of

Table 1. Effect of dexamethasone on the ratio of tyrosine hydroxylase activity to total biopterin in PC-12 cells.*

	Tyrosine hydroxylase activity		
	Total biopterin (% of zero-time value)		
Culture time (hr)	Control	Dexamethasone	Dexamethasone Control
24	120 ± 14	172 ± 15†	1.3
48	176 ± 28	231 ± 51†	1.3
72	149 ± 12	452 ± 50‡	3.0

* Values for tyrosine hydroxylase activity (nmoles L-DOPA/hr/mg protein) and total biopterin (ng biopterin/mg protein) derived from the experiments described in Figs. 1 and 3 were used to calculate the ratio of tyrosine hydroxylase activity to total biopterin in PC-12 cells. The ratio was calculated for each flask of cells, and means were taken of the results of three separate experiments in which four to five flasks were cultured at each time point. The results are expressed as a percentage of zero-time values. All ethanol control values were significantly greater ($P<0.05$) than their zero-time controls.

† $P<0.05$ vs ethanol control.

‡ $P<0.001$ vs ethanol control.

intracellular BH_4 concentration in the regulation of tyrosine hydroxylase activity, it has therefore been proposed that GTP cyclohydrolase and BH_4 levels in adrenal medulla may be subject to long-term regulation in conjunction with tyrosine hydroxylase, in order to allow full expression of changes in tyrosine hydroxylase levels [20].

The present study has examined total biopterin levels in PC-12 cells, an established line derived from rat pheochromocytoma, under conditions where tyrosine hydroxylase was induced by a synthetic glucocorticoid, dexamethasone. Exposure of PC-12 cells to dexamethasone for several days results in an induction of tyrosine hydroxylase protein [28–30] due to an elevation in the amount of tyrosine hydroxylase mRNA [30]. However, in the present study only very small, if any, changes in PC-12 cell total biopterin were observed following treatment of cells with dexamethasone for up to 3 days, despite a 2- to 3-fold induction of tyrosine hydroxylase. Since at least 95% of biopterin in control PC-12 cells was present as BH_4 or qBH_2, the absence of any marked increase in total biopterin in these cells indicates that PC-12 cell BH_4 levels were not increased following culture of the cells in dexamethasone. This finding therefore contrasts with the increased BH_4 levels in rat adrenal medulla and cultured bovine adrenal chromaffin cells under conditions where tyrosine hydroxylase is induced and the rate of tyrosine hydroxylation is increased [13, 17, 18].

One possible explanation for the apparent discrepancy between the results of the present study and those of Viveros and coworkers is that dexamethasone induction of tyrosine hydroxylase in PC-12 cells may not have been expressed as an elevation in the rate of tyrosine hydroxylation in intact cells. Whilst this was not measured in the present study, tyrosine hydroxylase induction by dexamethasone in PC-12 cells has been reported to be accompanied by a corresponding increase in intact cell catecholamine synthesis [29, 31]. Therefore, it is likely that in the present study the rate of tyrosine hydroxylation was also increased. However, it is interesting to note that there is at least one situation where tyrosine hydroxylase induction is not necessarily expressed as an increased rate of tyrosine hydroxylation. Seven days after reserpine administration, tyrosine hydroxylation in hippocampal and cerebellar synaptosomes was not elevated, despite an unmistakable induction of tyrosine hydroxylase as measured by increased enzyme activity in homogenate extracts [32]. Another possible explanation for the lack of effect of dexamethasone on biopterin levels is that a large induction of tyrosine hydroxylase may be required to produce changes in BH_4 levels. The increases in tyrosine hydroxylase and GTP cyclohydrolase activity in adrenal medulla and chromaffin cells always appear to be larger in magnitude than the increases in BH_4 levels. For example, a 60% increase in chromaffin cell BH_4 was observed when tyrosine hydroxylase activity and the rate of tyrosine hydroxylation were increased 2- to 3-fold, respectively [17], whereas in the present study, 2- to 2.5-fold increases in PC-12 cell tyrosine hydroxylase activity elicited very little, if any, increase in BH_4 levels. If PC-12 cell BH_4 levels are regulated in a manner similar to the coordinate regulation of tyrosine hydroxylase, GTP cyclohydrolase and BH_4 proposed for chromaffin cells, the mechanism must be less sensitive to alterations in tyrosine hydroxylase activity.

Another possibility which should be considered is that increased biopterin in the cells may have leaked into the medium rather than being retained by the cells and, therefore, have been undetected in the present study. However, total biopterin levels in the media of control PC-12 cells were only 22% of the amount in the cells and, therefore, reflect only a small proportion of biopterin in cells plus medium. Alternatively, BH_4 may have been released into the

medium and degraded to compounds other than biopterin, as had been demonstrated to occur in another culture medium, BGJ medium [33].

The molar ratio of tyrosine hydroxylase subunits and BH_4 may be an important parameter in the regulation of catecholamine synthesis. Our laboratory recently reported [10] that in rat striatal dopaminergic terminal there appears to be approximately equimolar amounts of tyrosine hydroxylase subunits and BH_4. In the current experiments if we assume the activity of tyrosine hydroxylase in cell extract is similar to the specific activity of pure tyrosine hydroxylase [34] derived from PC-12 cells, we can calculate that there is about 0.060 nmoles of tyrosine hydroxylase subunit per mg of protein in control cells. In these same cells there is about 0.066 nmole of BH_4 per mg of protein. Therefore, in control PC-12 cells there also is an approximate 1:1 ratio of tyrosine hydroxylase subunits and BH_4. However, if we look at this ratio in cells that have been exposed to dexamethasone for 72 hr, it appears to be 3:1. While there are many possibilities for error in these calculations, it is apparent that there is a large change in the ratio. What this means with regard to catecholamine synthesis in these cells remains to be determined.

Since BH_4 may represent a regulatory point in the tyrosine hydroxylase reaction, it would be important to know the BH_4 concentration in the cell. An approximate estimate can be made if we assume that 10% of the cell weight is protein and 85% is water. A typical value of BH_4 content is 17 ng/mg protein. Therefore, this is equivalent to 2 ng BH_4/mg cell water or 8.6 μM. This value is close to the K_m value of tyrosine hydroxylase in the activated state and far below the K_m of the non-phosphorylated enzyme. Since the concentration of tyrosine hydroxylase appears to be equal to or greater than BH_4, it is likely that the cofactor is predominantly associated with the activated form of the enzyme. Therefore, the rate of catecholamine synthesis may be more reflective of the content of activated enzyme rather than total enzyme.

In summary, PC-12 cell total biopterin levels were not increased significantly following culture of the cells in dexamethasone, despite an induction of tyrosine hydroxylase. Therefore, the molar ratio of tyrosine hydroxylase units to BH_4 was increased under these conditions. Studies of the effects of dexamethasone on catecholamine synthesis in these cells should clarify the role of BH_4 in the regulation of tyrosine hydroxylase activity.

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