Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 836-843

DEXAMETHASONE SELECTIVELY INCREASES MONOAMINE OXIDASE TYPE A IN HUMAN SKIN FIBROBLASTS

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Received December 30,1980

SUMMARY

The effects of several hormones known to affect monoamine oxidase activity in vivo have been studied in living human skin fibroblasts grown in culture. Of the hormones tested, the synthetic glucocorticoid dexamethasone caused the greatest increases in activity at physiologic concentrations. Increases of 10-12 fold were observed after 8-9 days of exposure to 5 x 10^{-8} M dexamethasone. This increase in activity was accompanied by a change in the relative proportion of the A and B types of activity in fibroblasts, from about 35% A:65% B in control cultures to 90% A:10% B in cultures exposed to dexamethasone. The increase in activity and the shift in the proportion of A and B activities could be accounted for almost exclusively by a specific increase in the number of Type A molecules.

INTRODUCTION

Catecholamines and serotonin exert numerous physiologic effects and influence a wide spectrum of behaviors (1). Changes in the metabolism of these biogenic amines have been observed in a number of neurologic and psychiatric disorders and may contribute to their pathology (2,3). Monoamine oxidase (MAO, E.C. 1.4.3.4) is primarily responsible for the degradation of biogenic amines and has a major role in determining levels of these neurotransmitters. The physico-chemical properties of MAO have been studied extensively in a variety of tissues in man and other mammals (4-6), but relatively little is known about how MAO activity is regulated. In this investigation, human skin fibroblasts were used as a model system to study the regulation of MAO activity in living cells. Skin fibroblasts can be grown in culture as homogeneous cell populations under controlled environmental conditions, and express both A and B types of MAO activity, also found in the nervous system (7,8). As a first attempt in understanding how physiologic factors control the expression of MAO, we studied whether changes in activity in fibroblasts could be mediated by hormones known to affect MAO in vivo (9,10).

ABBREVIATIONS: MAO, monoamine oxidase: DMEM, Dulbecco's Modified Eagle's Medium; FCS, fetal calf serum; PBS, phosphate buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; dex, dexamethasone.

0006-291X/81/030836-08$01.00/0

METHODS

Cell Culture. Human skin fibroblast lines HF22 and HF27 were established from minced explant cultures, as described (11,12). Monolayer cultures were grown in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO H-21) supplemented with 5% (v/v) fetal calf serum (FCS, Irvine Scientific) without antibiotics and fed at 6-8 day intervals (13), unless otherwise indicated. Lines were studied well before senescence to avoid changes in MAO which occur with aging in vitro (13; Edelstein and Breakefield, unpublished data). Stock cultures were grown in 75 cm^2 tissue culture flasks (Corning) and subcultured (1:8 split ratio) every 3-4 weeks by low temperature trypsinization (14). Cells for MAO assay were plated onto 16 mm multi-well cluster dishes (Costar). When monolayers were nearly confluent, cellular protein was labeled by growth of cultures for 4-8 days in fresh DMEM-5% FCS containing [^{14}C]leucine (0.1-0.2 μC_i/ml, 344 mC_i/mmol, NEN) and antibiotics (1:250 dilution of 100X antibiotic-antimycotic and Kanamycin solutions, GIBCO). Cells for electrophoretic analysis were plated onto 150 mm dishes (Falcon).

Hormone additions. Cultures were grown on dishes, as indicated above. Four to eight days after reaching confluency, the cell monolayer was rinsed twice with isotonic saline (14) and incubated in serum-free MCDB 105 medium (15) without antibiotics for 24 hr. Cultures were then exposed for varying lengths of time to fresh MCDB 105 containing the hormone of interest. Stock hormone solutions (5 mM) were prepared in absolute ethanol.

Monoamine oxidase assay. Activity in living cells was measured directly in cluster wells using tryptamine, a substrate for both MAO A and B (4-6). Monolayers were rinsed 3-4 times with isotonic phosphate buffered saline (PBS, 16) prior to incubation with fresh PBS containing 22.5 μM [G-^{3}H]tryptamine (1.3 μC_i, 1.5 C_i/mmol, Amersham Searle) and 3.7 μM ascorbic acid in a final volume of 350 μl (final pH 7.5). Reactions were carried out for 30-35 min at 37°C and stopped by the addition of 20 μl 10 N NaOH. The cells were dissolved by incubation for 1-2 hr at 25°C. Lysates were acidified by the addition of 30 μl 10 N HCl, and 200 μl of this mixture were added to a 1 dram shell vial (Rochester Scientific), followed by the addition of 4 ml toluene containing 4.2% Liquifluor (NEN). After shaking 5-10 min to extract deaminated products and protein into the toluene layer, the entire vial was counted in a Beckman liquid scintillation spectrometer. Background values were established by incubating cell monolayers with clorgyline (0.1 mM, 5-15 min, 25°C) prior to assay. Unless otherwise indicated, only relative specific activities were calculated using the ratio of [^{3}H]cpm (corrected for background) to [^{14}C]cpm obtained from each cluster well. To calculate actual specific activities (pmol/min/mg protein), protein was measured in 3-4 cluster wells and the ratio of [^{14}C]leucine cpm to mg protein was determined. Under the conditions used, [^{14}C]leucine incorporation was directly proportional to protein concentration. To determine drug sensitivity, cell monolayers were incubated with varying concentrations of clorgyline or deprenyl for 30 min at 37°C prior to assay.

[^{3}H]Pargyline binding to crude mitochondrial proteins. Cultures were harvested from 150 mm dishes using PBS, and crude mitochondrial fractions were prepared immediately, as described (17). Freshly prepared mitochondrial fractions (0.7-1.1 mg protein/ml) were incubated for 2 hr at 37°C with 1-1.5 μm [^{3}H]pargyline (1.7-2.8 μC_i, 6.86 C_i/mmol, NEN) in a final volume of 350-400 μl 50 mM Tris-HCl, pH 7.5. Following binding, samples were diluted with 5 ml Tris buffer and centrifuged at 40,000 g for 60 min at 4°C. The mitochondrial pellet was stored at -20°C.

Electrophoretic analysis. Labeled mitochondrial pellets (125 μg) were resuspended in 80 μl loading solution, proteins were solubilized, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for 24-26 hr at 45 V, and gels were stained, destained and prepared for fluorography, as described (18). Dried gels were exposed to preflashed Kodak X-Omatic R film for 2 months at -70°C and fluorograms were analyzed by densitometry using a Beckman DU-8 spectrophotometer.

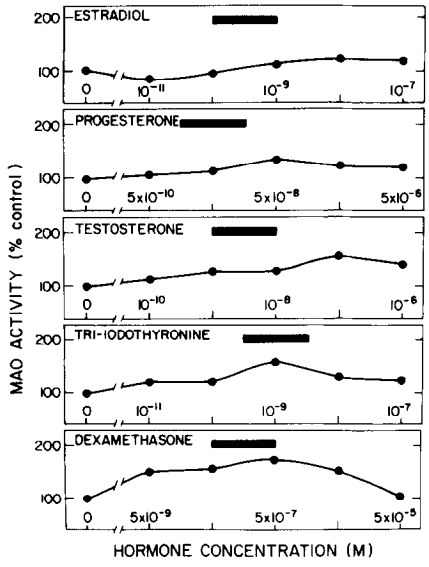
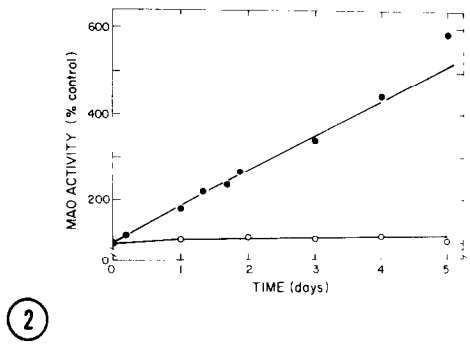


Figure 1: Effects of varying hormone concentrations on MAO activity. Post-confluent cultures in multi-well cluster dishes were exposed for six hours to serum-free MCDB 105 medium containing varying concentrations of each hormone. The range of physiologic concentrations for each hormone is indicated by the bar above each curve (see 20). MAO activity was measured in living cells using [G-^{3}H]tryptamine as a substrate and protein content was determined by [^{14}C]leucine incorporation (see Methods). Results are plotted as the percent of activity (i.e. [^{3}H]/[^{14}C] ratio) in control cultures not exposed to hormone.

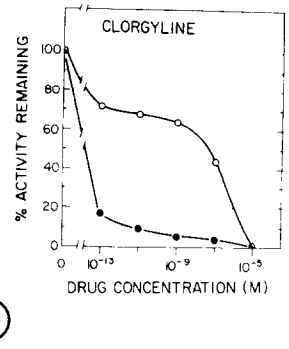
Protein determination. Protein concentrations were determined by the method of Bradford (19) using bovine serum albumin as a standard.

RESULTS

Effect of various hormones on MAO activity. Several hormones known to affect MAO activity in vivo (9,10) were tested for their effects on MAO activity in living fibroblasts in culture. The results of a six hour exposure to varying concentrations of these hormones are shown in Fig. 1. Estradiol had a biphasic effect on MAO activity. Low concentrations (10^{-11}M) caused a 15% decrease in activity, while higher concentrations (10^{-8} to 10^{-7}M) increased activity 20-25%. Progesterone and testosterone increased MAO activity, with optimal effects at slightly higher than physiologic concentrations. Progesterone increased MAO activity almost 40% at 5 x 10^{-8}M and testosterone increased activity nearly 60% at 10^{-7}M. In contrast to the sex steroids, the thyroid hormone tri-iodothyronine caused optimal increases in activity at physiologic concentrations, almost 60% at a concentration of 10^{-9}M. Of all the hormones tested, the synthetic glucocorticoid dexamethasone (dex) had the greatest effect on MAO activity at concentrations physiologic for glucocor-



(2)



(3)

Figure 2: Time course of dex response. Post-confluent cultures were exposed to serum-free medium with and without 5 x 10^{-8}M dex and MAO activity was measured periodically over five days, as described in Fig. 1. Activity in control cultures (o, open circles) was 61 pmol/min/mg protein. Results for dex treated cultures (•, closed circles) are plotted as the percent of control activity (i.e. $[^{3}H]/[^{14}C]$ ratio) at each time point.

Figure 3: Sensitivity of MAO activity to inhibition by clorgyline. Post-confluent cultures were exposed to serum-free medium with and without 5 x 10^{-8}M dex for 7-8 days. Monolayers were preincubated for 30 min at 37°C with varying concentrations of clorgyline prior to measurement of MAO activity, as described in Fig. 1. Results are plotted as the percent of activity remaining; 100% activity (no clorgyline) was 57 pmol/min/mg protein in control cultures (o, open circles) and 685 pmol/min/mg protein in dex-treated cultures (•, closed circles).

ticoids, with an optimal increase in activity of nearly 80% at 5 x 10^{-7}M. All further experiments, therefore, concentrated on the effects of dex on MAO activity using a concentration of 5 x 10^{-8}M which was found to be optimal in subsequent studies (data not shown).

Time course of dex response. The results of exposing cultures to dex for various periods of time are shown in Fig. 2. MAO activity at the start of the experiment was 61 pmol/min/mg protein. In cultures not exposed to hormone, protein content and MAO levels remained constant throughout the five days. In parallel cultures exposed to dex, there was a linear increase in activity with time while protein content remained constant. No apparent lag was seen, even at times as short as six hours, and increases of 5-6 fold were observed after a five day exposure to the hormone. When cultures were exposed to dex for 8-9 days, increases of 10-12 fold were found (data not shown).

Alterations in drug sensitivity. In order to determine whether exposure to dex increased both types of MAO activity, the relative proportions of MAO A and B activities in living cells were determined by measuring the sensitivity of tryptamine deamination to selective MAO inhibitors. Dose response curves for clorgyline, a selective A inhibitor (4-6), are shown in Fig. 3. In control

cultures, approximately 35% of the total activity was Type A (i.e. inhibited by 10^{-9}M clorgyline) while 65% was Type B (i.e. inhibited by 10^{-5}M clorgyline). In parallel cultures exposed to dex for 7-8 days, activity increased 12-fold, from 57 to 685 pmol/min/mg protein. Approximately 90% of the total activity was now Type A and only 10% was Type B. When the selective Type B inhibitor deprenyl (4-6) was used, a similar change in the relative proportion of A and B activities was seen after treatment with dex (data not shown).

Electrophoretic analysis. Radiolabeled pargyline can be used to detect MAO by SDS-PAGE (17,21,22) since this inhibitor binds specifically to the enzyme, forming a covalent adduct with the flavin cofactor (23). Because the labeled flavin polypeptides associated with MAO A and B types of activity can be separated by SDS-PAGE (18,24,25), and because [^{3}H]pargyline binds stoichiometrically to the flavin polypeptide (23), the relative amounts of MAO A and B can be quantitated by determining the amount of radioactivity in each of the labeled bands. No major differences in the Coomassie blue banding patterns of the mitochondrial proteins were observed between control and dex treated cultures (Fig. 4, Lanes A and B). Two [^{3}H]pargyline labeled bands with apparent molecular weights of 63,000 and 60,000 daltons, representing MAO A and B, respectively (18), were detected by fluorography (Fig. 4, Lanes D and E). Hormone treatment did not alter the electrophoretic mobilities of these labeled flavin polypeptides but did affect their amount. Quantitation of MAO A and B in control and dex treated samples was determined by densitometer analysis of the fluorogram shown in Fig. 4B. After exposure to the hormone for eight days, the amount of labeled MAO A flavin polypeptide increased over 15-fold while that of MAO B increased only three-fold.

DISCUSSION

Numerous studies have shown that hormones can alter levels of MAO activity in vivo. However, depending on the age and sex of the animal, as well as the tissue examined, the same hormone can increase or decrease activity (9,10). The present findings indicate that, as in vivo, hormones can regulate MAO activity in living human skin fibroblasts grown in culture. Of the hormones tested, including the thyroid hormone tri-iodothyronine and the sex steroids estradiol, progesterone and testosterone, the synthetic glucocorticoid dex caused the greatest increases in MAO activity. Exposure to "physiologic" concentrations of dex resulted in a linear increase in MAO activity with time, with increases of 80% after a six hour exposure and 10-12 fold after 8-9 days of hormone treatment.

Increases in total MAO activity were accompanied by a change in the relative proportion of A and B activities in cultures exposed to dex, suggesting

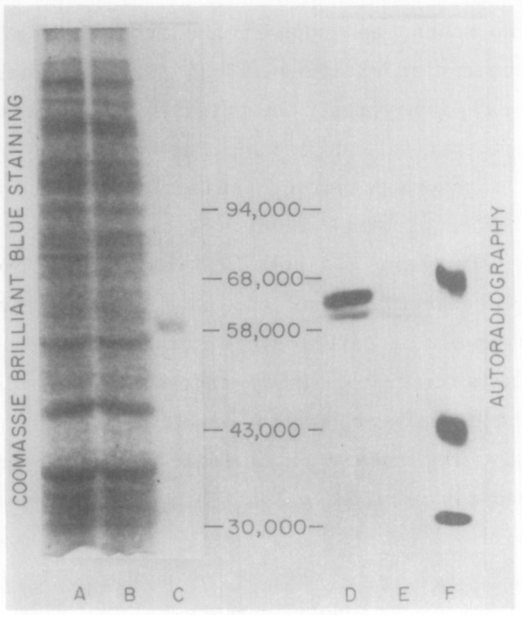


Figure 4: SDS-PAGE of [^{3}H]pargyline labeled MAO. Crude mitochondrial fractions were prepared from post-confluent cultures grown on 150 mm tissue culture dishes and exposed to serum-free medium with and without 5 x 10^{-8} M dex for eight days. Samples were incubated with [^{3}H]pargyline for 2 hr at 37°C, solubilized and subjected to SDS-PAGE (see Methods). (A-C) Coomassie brilliant blue protein staining pattern; (D-F) autoradiographic banding pattern of the same gel lanes; (A and D) samples from dex-treated cultures; (B and E) samples from control cultures; (C) catalase; (F) bovine serum albumin, ovalbumin, carbonic anhydrase.

that the increase in total activity resulted from a specific increase in MAO A activity. To examine this possibility, [^{3}H]pargyline was used to radiolabel and quantitate the flavin polypeptides of MAO A and B. The increases in MAO activity and the corresponding shift in the proportion of A and B activities after exposure to dex could be accounted for almost quantitatively by a specific increase in the number of A molecules. Although a small increase in the number of B molecules was observed in this study, such increases are not consistently found (data not shown).

Specific changes in levels of MAO A are not unique to glucocorticoid treatment of cultured cells. Administration of 17 β-estradiol can lead to an enhanced degradation of MAO A in the brains of ovariectomized rats (26). Similarly, neonatal castration or androgen administration can change MAO A activity in rat liver (27). Further, increases in endogenous levels of progesterone can result in a rise of MAO A activity in the human and rat female genital tract (28).

These studies indicate that the levels of MAO A can be regulated independently of MAO B by hormones, and support the accumulating evidence that MAO A and B are separate molecular entities (18,24,25,29). Hormone-induced changes in MAO A activity may have important physiologic effects since neurotransmitters such as norepinephrine and serotonin are preferred substrates for MAO A (4-6). In a number of psychopathologic states associated with altered levels of MAO activity (30-32), abnormal levels of circulating glucocorticoids have been noted (33-35). Further, patients who have been treated by exogenous administration of glucocorticoids or who have altered levels of circulating glucocorticoids as a result of pathological changes in the adrenal glands (e.g. Addison's and Cushing's disease) diplay psychological manifestations (34-36) similar to the psychopathologic states associated with altered MAO levels. Such findings strongly implicate a physiologic role for the regulation of MAO by glucocorticoids and may provide insight into means by which MAO activity may be regulated in vivo.

ACKNOWLEDGEMENTS

We thank Richard Cawthon for advice and help in analyzing MAO by SDS-PAGE, Carmela Castiglione and Virginia Bonnefil for assistance with cultures, and Nancy Dillon for typing this manuscript. This work was supported by USPHS grants NS12105 and GM20124.

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