CATECHOLAMINES IN STRESS: MOLECULAR MECHANISMS OF GENE EXPRESSION

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The catecholamines play key roles in orchestrating the response to stress. While this is crucial to handle emergency situations, stress becomes maladaptive when prolonged or repeated, increasing allosteric load and susceptibility to a wide range of serious diseases. The time frame of the regulation of gene expression, especially as it relates to catecholamine (CA) biosynthetic enzymes are compared in three crucial catecholaminergic locations, the adrenal medulla, sympathetic ganglia and locus coeruleus in male animals. The adrenal medulla displays very rapid response to stress and gene profiling reveals a wide repertoire of target genes, many of them activated by single and not by repeated stress. In contrast to the adrenal medulla, the sympathetic ganglia are especially responsive to activation of the HPA axis, and ACTH may have a direct effect. The locus coeruleus, origin of most of the noradrenergic neurons innervating much of the brain, displays activation of additional signalling pathways and transcription factor with repeated compared to single exposure to stress.

Most of the studies have been performed in males. However, there is considerable evidence that females respond differently to stress. Estradiol can regulate TH, DBH and GTPCH gene expression, as well as to modulate its response to other second messenger such as cAMP. Prior treatment with estradiol was found to alter the response of CA biosynthetic enzymes to stress.

This emphasizes the tissue and sex specific features of the mechanistic underpinning of the adaptation or maladaptation of the catecholaminergic systems to stress and provides the basis for specific interventions.

Keywords: Adrenal medulla – Catecholamine biosynthesis – Estrogen – Sympathetic ganglia – Locus coeruleus – Stress – Transcription factors

Introduction

It is a great honor to be invited as introductory lecturer for the 9th Symposium on Catecholamines and Other Neurotransmitters in Stress. These Smolenice Castle meetings, of which this my fifth, have been such a wonderful venue for high caliber science, lively discussions, planned collaborations and enjoyment of the lovely Slovak countryside with exceptional hospitality. Most of the participants savor the opportunity for renewed collaborations and friendships that is not to be missed. Where else can you have Julius Axelrod help interpret and plan your latest experiments while enjoying traditional Slovak music and dance around a campfire. It is humbling to follow the outstanding previous introductory lectures which were presented by Julius Axelrod (1991), David Goldstein (1995), Miklos Palkovits (1999) and E. Ronald DeKloet (2003).

In reflecting on my twenty-five years of research on molecular biology of the catecholaminergic systems, and more than 15 as it relates to stress, it is remarkable how much has been accomplished. Nevertheless, stress remains one of the most serious health issues, which is even more critical with worldwide terrorism. Thus, it is striking how much still remains to be done.

Stress, whether physiological, psychological or environmental, is a two-pronged phenomenon. On one hand, it has an important adaptive function that promotes improved health and survival by enabling the individual to overcome emergency situations. On the other hand, stress can be extremely harmful when excessive (SELYE 1975). In particular, an individual who is unable to cope with stress is highly susceptible to a variety of diseases. However, when the HPA axis, the sympathoadrenal and central CA systems are activated repeatedly over a long period of time the response is not only adaptive, but also maladaptive [reviewed in (CHROUSOS and GOLD 1992; MCEWEN 1998)]. Prolonged stress increases the allosteric load (MCEWEN and STELLAR 1993; SEEMAN et al. 1997) and is a major contributor to the development of cardiovascular disorders and to psychiatric illnesses. Stress also increases the body's susceptibility to infection, autoimmune diseases, chronic fatigue syndrome, and cancer. Many separate studies have confirmed that stress increases the propensity of an individual to self-administer drugs of abuse (PIAZZA and LE MOAL 1998). Moreover, stress can influence the progression of chronic diseases. For example, stress adversely affects the maintenance of appropriate blood glucose levels in diabetics. In fact, it has been proposed that nearly two-thirds of ailments seen by physicians are either stress-induced or stress-related.

I would like to take this opportunity to summarize a comparison of the regulation of gene expression in catecholaminergic systems, especially as it relates to CA biosynthetic enzymes in three crucial catecholaminergic locations, the adrenal medulla, sympathetic ganglia and locus coeruleus in male rats, and finish with some intriguing findings relating to effects of estrogen and sex differences in stress related disorders.

MOLECULAR REGULATION OF CATECHOLAMINERGIC PATHWAY BY STRESS IN VARIOUS CATECHOLAMINERGIC LOCATIONS

1. Adrenal Medulla

The epinephrine (Epi), primarily from adrenal medulla and norepinephrine (NE), from sympathetic nerve endings and adrenal medulla, activate the heart and skeletal muscle for the "fight or flight" response to acute stress. In addition, adrenal catecholamines, and especially Epi, plays a key role in memory retention and retrieval (MCCARTY and GOLD 1981; BORRELL et al. 1983; HALL and GOLD 1990).

The release of catecholamines from the adrenal medulla is among the most rapid responses to stress. In the 70s, Richard Kvetnansky and co-workers determined that the stress triggered release of Epi and norepinephrine NE from the adrenal are so rapid that animals must be cannulated in order to obtain an accurate measure of the rise in plasma NE and Epi (KVETNANSKY et al. 1978). The sustained release of CAs leads to reduced adrenal Epi levels. However after prolonged daily exposure to IMO for one week or over one month, adrenal medullary NE and Epi attain new elevated levels, with adrenal Epi about 50 % higher and NE nearly double basal level (KVETNANSKY and MIKULAJ 1970). This was subsequently shown to result increased activity of catecholamine biosynthetic enzymes (see Fig. 1 for pathway of catecholamine biosynthesis) (KVETNANSKY et al. 1970; 1971b). For example, repeated exposure to IMO lead to elevated TH and DBH protein levels and enzymatic activity, while no significant change is observed with a single exposure to this stressor (KVETNANSKY et al. 1970, 1971a; SABBAN and KVETNANSKY 2001).

Therefore, it is surprising that as early as 5 min exposure to a single exposure to IMO (shortest time examined) there is a already a 3-4 fold increase in TH and DBH transcription rate. This elevation in transcription is as high as observed with continual 2 hrs IMO or with repeated exposure to IMO stress (NANKOVA et al. 1999). Thus transcription of TH and DBH genes are almost as fast as the stress triggered rise in adrenal glucocorticoids.

What are some of the other very rapid responses? A time line for the response of the adrenal medulla to an acute exposure to IMO is shown in Fig 2. Five minutes of IMO is also sufficient to activate the two MAP kinases examined, ERK1/2 and JNK. The activation of ERK1/2 is transient, elevated after 5 min but not longer (SABBAN et al. 2006), while JNK was sustained at least 30 min (longest time examined) (NANKOVA et al. 1998).

We have examined phosphorylation of CREB, a key transcription factor integrating a variety of signalling pathways, including those involving cAMP- and calcium- dependent kinases, and MAP kinases [reviewed by

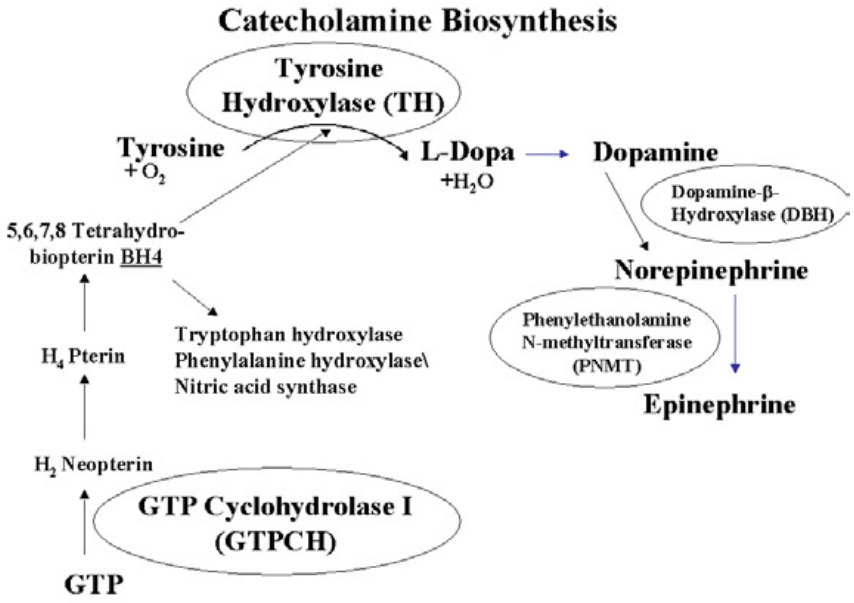

Fig 1 Pathway for catecholamine biosynthesis, including biosynthesis of tetrahydobiopterin, essential cofactor for TH.

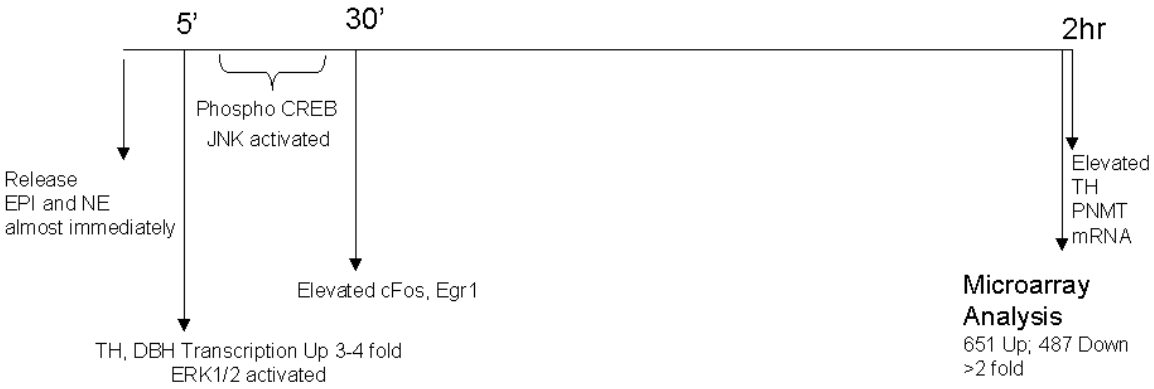

Fig 2 Time line of response of adrenal medulla to acute IMO stress.

(MAYR and MONTMINY 2001)]. Phosphorylation of CREB (Phospho-CREB) on serine residue needed for transcriptional activation begins to become apparent with already 5 min of IMO, and is more pronounced after 30 min. It may be mediated by the rapid activation of ERK. However, by 2 hrs of single IMO, levels of phospho-CREB returned to basal levels, reflecting the transient nature of this response (SABBAN et al. 2006).

By the end of 2 hr of IMO stress, TH and PNMT mRNAs are highly elevated (MCMAHON et al. 1992; VISKUPIC et al. 1994), although greater levels are observed 3 hr after cessation of the stress (NANKOVA et al. 1994; WONG et al. 2002). The higher level of DBH transcription is not maintained for the entire 2 hr period. DBH mRNA does not obtain maximal levels with single IMO.

One of the main features of changes in gene expression for CA biosynthetic enzymes with single exposure to IMO stress is that with single IMO the elevation of transcription and rise in mRNA levels is tran-

sient (NANKOVA et al. 1994; OSTERHOUT et al. 1997; NANKOVA et al. 1999). However, even with only a second exposure to the same stressor, there is 'memory' of the first experience such that the increase in TH mRNA is now much more prolonged and is sustained for longer periods of time after termination of the stress. With prolonged repeated IMO stress over 5-7 days, the elevation of mRNA levels in the adrenal medulla remains high for long periods of time following stress termination (NANKOVA et al. 1994; VISKUPIC et al. 1994). The transcription rate is also more sustained (NANKOVA et al. 1999) and TH transcription remains high for as long as 2 days following cessation of repeated daily IMO stress for seven days (SUN et al. 2003). Consistent with this, early studies showed that the excess TH activity in the adrenals of rats repeatedly stressed for one week declines toward basal levels with first order kinetics and a half life of three days (KVETNANSKY et al. 1970).

2. Global profiling: 2 hr IMO once or repeatedly

Microarray profiling was used to determine global changes in gene expression in the adrenal medulla and to reveal common and distinct response to single and repeated stress. Rats were exposed to 2 hr IMO once or repeatedly for six consecutive days. Gene profiling (RAE 230 2.0 Affymetrix) covering the entire rat genome was used to elucidate stress triggered changes in gene expression (LIU et al. 2006). We concentrated on the changes which differed highly significantly ($p<0.0$) and were of up- or down- regulated greater than 2-fold compared to unstressed controls. The largest number of genes were significantly changed ($p<0.01$) by single IMO - with 651 up- and 487 down-regulated. With repeated IMO, 370 were up- and 195 were down-regulated. Undefined genes which were removed from further analysis.

We organized and categorized all the defined genes based on Gene Ontology and PubMed publications. The percent of the changes in the various categories is shown in Figure 3. The largest number of changes was observed in transcription factors with both single and repeated stress. A substantial number of changes were also observed in growth factors related transcripts, especially after even a single exposure to IMO. Other prominent categories were metabolism, including lipid metabolism, protease and kinase/phosphatase related genes. With repeated IMO, a higher percentage of transcripts are devoted to secretion neuropeptide related genes.

Most of the genes (>80 %) altered by single IMO were unique indicating that they are transiently elevated. In contrast, approximately half of the defined genes elevated by repeated stress were also responsive to single IMO indicating that they mediate a prolonged response.

One of the key surprising findings in analyzing the global changes how profound and extensive are the changes in gene expression with even a very brief exposure to stress.

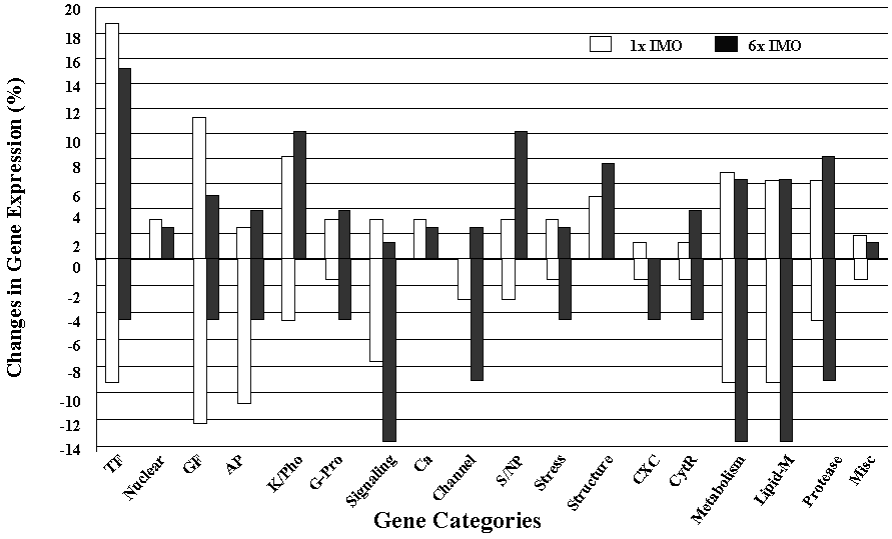

Fig 3 Categorization of the changes in gene expression observed in microarray analysis after IMO for 2 hr of once (1 x IMO) or for 6 consecutive days (6 x IMO).

3. Sympathetic Nervous System

Many studies have analyzed the relationship between sympathetic nerve activity, plasma NE and hypertension. Plasma NE levels (primarily from sympathetic nerve endings, but also from adrenal medulla) are also markedly elevated with aging in humans and experimental animals, and has been proposed to be involved with increased hypertension in the elderly [reviewed by (GOLDSTEIN 1995)]. NE released from sympathetic nerves generally contract smooth muscle cells, eliciting glandular secretion, vasoconstriction and myocardial contraction. The systemic vasoconstriction increases total peripheral resistance to blood flow in the body, which combined with the myocardial stimulation, increases blood pressure. Overall, it was concluded that sympathetic efferent nerves play an important role in the control of blood pressure in a number of situations. They appear to maintain hypertension, even when they are not the primary initiating cause. However their role in initiation or maintenance of essential hypertension is still controversial [reviewed by (MATHIAS 1991)].

Changes in sympathoneural function associated with myocardial infarction can be primary, with augmented cardiac or extracardiac NE release increasing myocardial oxygen consumption and exacerbating ischemia, or can be secondary, with recruitment of cardiac and extracardiac sympathoneural outflows maintaining cardiovascular performance [reviewed by (GOLDSTEIN 1995)].

Congestive heart failure is also associated with activation of the sympathetic nervous system. While this activation may provide short term hemodynamic support to the failing heart, over long periods of times it is deleterious. Preclinical and clinical studies suggest that the chronic sympathetic activation in congestive heart failure is a maladaptive response which accelerates the progressive worsening of the disease. Pharmacological approaches to block sympathetic activation are used to treat heart failure. These include inhibition of central sympathetic outflow, block of cardiac effects (b-adrenergic blockers) and recently inhibition of CA biosynthesis [reviewed by (KRUM 1999)]. Recent studies have used inhibitors of DBH for congestive heart failure. In dogs with heart failure the DBH inhibitor, nepicastat was found to prevent the progression of left ventricular dysfunction and remodeling and prevent progressive worsening of cardiac function (HEGDE and FRIDAY 1998; SABBAH 2000).

Several lines of evidence indicate that the mechanism of stress triggered activation of TH and DBH gene expression differ in sympathetic nervous system and in the adrenal medulla reviewed by SABBAN et al. 2004). Among the most intriguing differences are in the response to ACTH injections. Regulation of TH and DBH (in contrast to PNMT) gene expression in the adrenal medulla with stress is not very much affected by the HPA axis, and increased TH and DBH expression is still observed in hypophysectomized animals (KVETNANSKY 1973).

Thus it was not surprising that injections of ACTH did not alter TH or DBH mRNA levels in the adrenal medulla. However, injection of ACTH triggered as large a rise in TH mRNA in superior cervical ganglia (SCG) as observed with IMO stress, indicating the importance of the HPA axis for regulation of the sympathetic nervous system (NANKOVA et al. 1996).

Administration of ACTH elicits an increase in blood pressure in normotensive and hypertensive subjects and is believed to be mediated by the ACTH triggered elevation of cortisol (WHITWORTH et al. 1983).

We postulated that ACTH may have a direct effect on expression of CA biosynthetic enzymes in sympathetic ganglia. To test this hypothesis we determined expression of ACTH (MC2) receptor mRNA and found it expressed in rat SCG as well as stellate ganglia (NANKOVA et al. 2003; SABBAN et al. 2004). In addition IMO stress found to increase MC2 receptor mRNA levels in the SCG.

To further examine the possible direct role of ACTH, independent of adrenal hormones on gene expression of NE biosynthetic enzymes in SCG, rats were subjected to bilateral adrenalectomy. Previous studies by Richard Kvetnansky, Karl Pacak, Irv Kopin, David Goldstein and coworkers showed that while adrenalectomy, as expected, nearly eliminated circulating epinephrine and increased plasma ACTH, it triggered markedly increased plasma NE levels. Furthermore, adrenalectomized rats displayed an exaggerated elevation of plasma NE and its metabolites when exposed to stress (KVETNANSKY et al. 1993). An increased urinary output of NE after bilateral adrenalectomy was also observed in humans (VON EULER 1954).

Following 12 days of bilateral adrenalectomy, or sham surgery, we determined plasma ACTH levels, as well as TH, DBH and MC2 receptor mRNA levels in SCG. As shown in Fig. 4 in adrenalectomized animals there was an elevation of both TH and DBH mRNA levels. This suggest that the exaggerated response of NE to stress may reflect from increased NE synthesis and that an intact adrenal gland is not required for this effect.

4. Locus Coeruleus

The LC is comprised of about 3000 neurons (both sides) in the rat and about 24,000 neurons in humans. These neurons comprise a broad network of projections that extend throughout the neuroaxis and account for about 70 % of all brain NE in primates (Foote et al. 1983). Ascending NE axons originating within the LC richly innervate numerous regions implicated in stress responses, including the extended amygdala, hippocampus, and prefrontal cortex, areas considered critical in mediating the alertness, focus and many other cognitive and physiological changes necessary in dealing with stress (Foote and Aston-Jones 1995). Acute

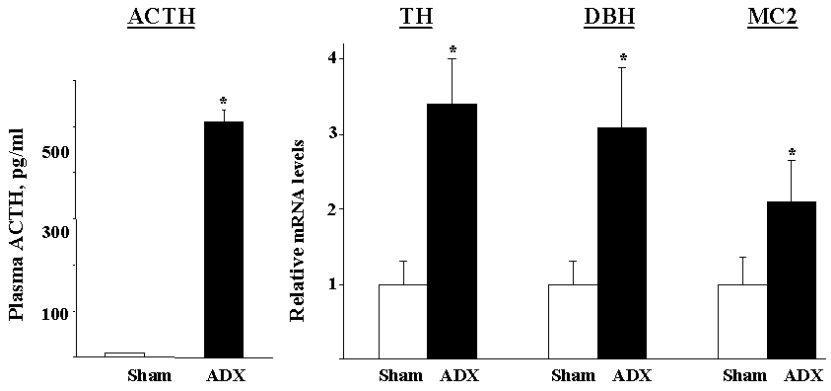

Fig 4 Effect of adrenalectomy on NE biosynthetic enzymes and ACTH Receptor in superior cervical ganglia. Rats underwent bilateral adrenalectomy (ADX) or sham operation and 12 days later levels of ACTH in plasma, TH, DBH and MC2 receptor mRNA were determined.

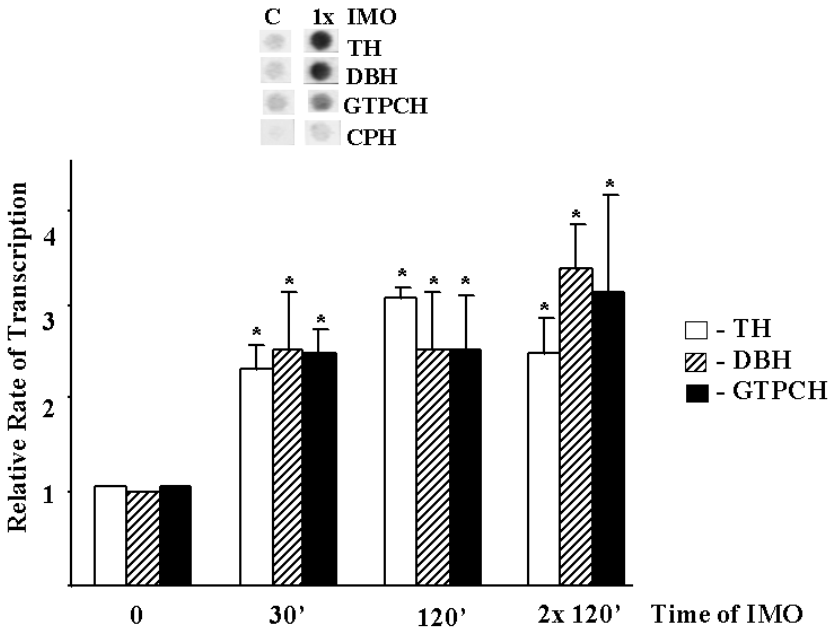

Fig 5 Both single and repeated immobilization stress induced transcriptional activation of TH, DBH and GTPCH genes. Rats were exposed to 0, 30, or 120 min of IMO stress once, or 120 min each on two consecutive days. Relative rates of transcription in the LC were determined by run on assays of transcription [from (Serova et al. 1999)].

stress releases NE in the terminal fields of the LC projections leading to a depletion of NE and an increase in NE metabolites (TANAKA et al. 1982; GLAVIN et al. 1983; SMITH et al. 1991). During repeated stress, brain NE levels are replenished by increased biosynthesis (WEISS et al. 1975; KVETNANSKY et al. 1977).

Many types of stress were found to trigger and elevated TH activity and protein levels in the LC. These include isolation footshock, restraint or immobilization, chronic social stress, forced walking and chronic cold. In addition, several studies also examined changes in DBH activity or protein levels and found them elevated by stress [reviewed by (KVETNANSKY and SABBAN 1993; SABBAN and KVETNANSKY 2001)].

The stress triggered increase in activity of NE-biosynthetic enzymes is accompanied (and in some cases preceded) by changes in their respective mRNAs (MAMALAKI et al. 1992; WATANABE et al. 1995; RUSNAK et al. 1998; WANG et al. 1998; SEROVA et al. 1999; HEBERT et al. 2005).

Elevations in transcription is implicated in mediating the stress triggered increased TH, DBH as well as GTPCH gene expression in the LC. As shown in Fig 5, we have found that both single and repeated IMO stress triggered about a 3-fold increase in levels of transcription initiation as determined by run on assays of transcription. Footshock also increased TH transcription in LC, as determined by a different method (in situ hybridization with introns specific probes) (CHANG et al. 2000). However, it appears that the elevation of TH transcription by stress in the LC is not nearly as high as occurs in the adrenal medulla (OSTERHOUT et al. 2005). Moreover the increased transcription, at least for TH, is not sustained very long after cessation of the stress, and post-transcriptional mechanisms are likely important to maintain the prolonged rise in mRNA levels in LC following repeated stress (SEROVA et al. 1999; SUN et al. 2004).

Induction of AP1 factors, such as c-Fos, Fra-2 and Jun family members can regulate TH transcription at the AP1 like motif. Stress rapidly induces c-Fos in the LC, and we and others have shown that while in many brain areas, induction of c-Fos is characteristic of acute activation, in the LC repeated stress continues to induce c-Fos expression. Our data with c-Fos deficient animals suggests that the induction of c-Fos may be essential for induction of gene expression of NE-biosynthetic enzymes (at least DBH) (SEROVA et al. 1998). Induction of Fra-2 in the LC was observed in our studies with repeated stress (HEBERT et al. 2005).

Phosphorylation of CREB, as well as induction of CREB levels, are observed with single and especially repeated IMO stress, and can activate TH transcription at the perfect consensus CRE motif. The role of cAMP pathway transcription factor CREB in regulation of gene expression in the LC has received considerable attention in a series of elegant studies [reviewed by (NESTLER et al. 1999)]. Chronic application of stress (or of an opiate such as morphine) upregulates the cAMP pathway in LC neurons, apparently with CREB involvement (MELIA et al. 1992; NESTLER 1992; NESTLER and AGHAJANIAN 1997). Thus, infusion of antisense oligonucleotide to CREB, which reduced basal CREB and TH levels by about 20 %, blocked the morphine induced up regulation of TH as well as spontaneous LC firing and development of physical dependence (LANE-LADD et al. 1997). While other CREB/ATF family members can also act on the CRE motif, we did not observe changes in ATF-2 in the LC with single or repeated IMO stress.

Although the Egr1/Sp1 is an important functional motif, Egr1 was not induced by repeated IMO stress in the LC.

Multiple MAP kinases are activated by chronic stress in the LC (HEBERT et al. 2005). Repeated immobilization stress was found to lead to increased phosphorylation of p38, JNK1/2/3 and ERK1/2. ERK1 was the major isoform expressed, and ERK2 was the predominant isoform phosphorylated. The phosphorylated ERK1/2, was localized selectively in TH immunoreactive neurons (Fig. 6).

These distinct alterations in transcriptional pathways following repeated, compared to single stress, may be involved in mediating long lasting neuronal remodeling and are implicated in the mechanisms by which acute beneficial responses to stress are converted into prolonged adaptive or maladaptive responses.

Our model is that the induction of c-Fos, perhaps by activation of cAMP/CREB, is necessary for the initial elevation of TH and DBH gene expression in LC. These responses are not attenuated, but are amplified with repeated stress with increased CREB expression, activation of several MAP kinases and induction of Fra-2. This enhances the chronic activation of the LC with profound, ultimately detrimental consequences.

5. Modulation by Estrogen

There are many indications that women respond differently than males to stress. For example, with regard

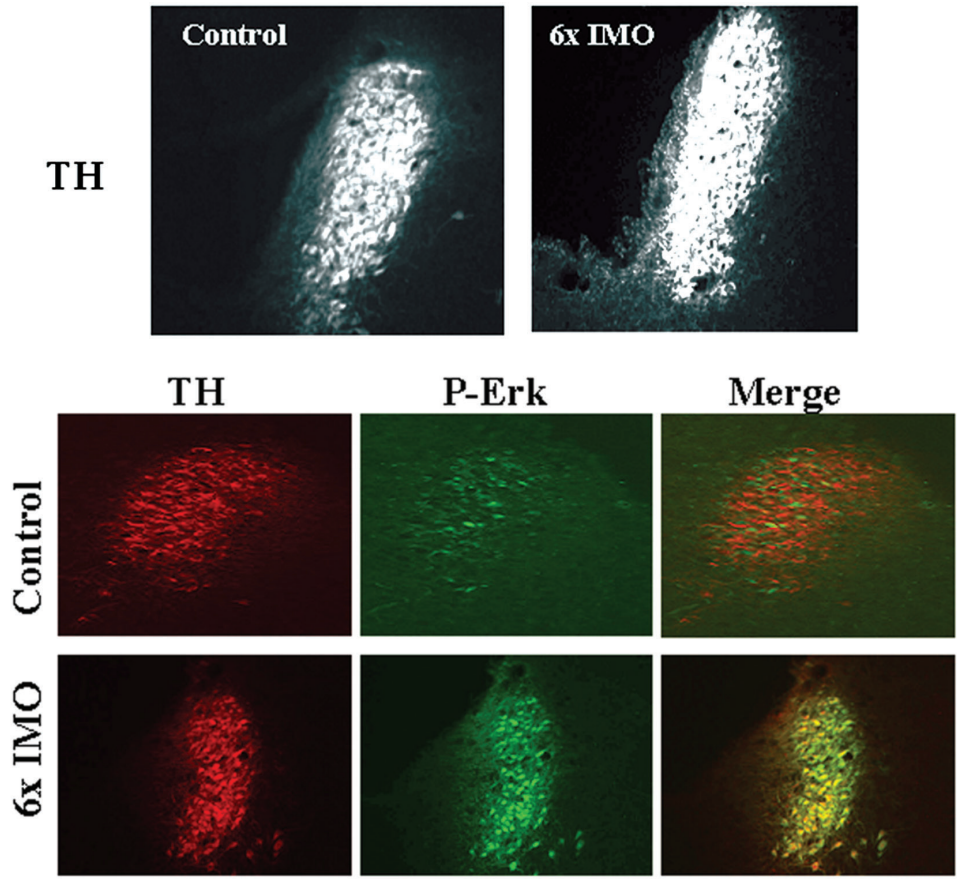

Fig 6 Effect of repeated immobilization stress on expression of TH and phosphorylation of ERK in the LC. Immunocytochemistry with antisera to TH or to phospho-ERK (P-ERK) in LC of control rats or rats exposed to IMO stress for 2 hrs daily on 6 consecutive days (6 x IMO) [from (HEBERT et al. 2005)].

to the HPA axis, females have higher basal and stress induced levels of ACTH and corticosterone than males (KITAY 1961; CRITCHLOW et al. 1963). Ovariectomy (OVX) of adult rats reduce plasma ACTH and corticosterone levels, and replacement of estradiol returns both hormones to control levels (BURGESS and HANDA 1992). In women, cortisol levels are significantly decreased after menopause but can be restored with estrogen replacement therapy (HELGASON et al. 1981). In addition, comparing responses to mental stress in pre- and postmenopausal women revealed an increased response after menopause, while estrogen therapy to postmenopausal women attenuated responses to mental stress (LINDHEIM et al. 1992; OWENS et al. 1993).

In perimenopausal women estrogen replacement therapy reduced total body NE spillover, an index of sympathetic neuronal activity (SUDHIR et al. 1997). Gender differences in regulation of NE release and uptake was found in sympathetic nervous system. The sympathoadrenal system in female rats are more reactive than that in males to the novel environment and footshock stresses (WEINSTOCK et al. 1998). For example, chronic restraint stress impairs radial maze performance in male rats, but enhances it in females. Attenuated stress-induced increases in plasma Epi in women suggest that females are less sensitive and/or less responsive to adrenal medullary activation (HINOJOSA-LABORDE et al. 1999).

Sex differences are found in the morphology of human and rat LC. Ascending NE axons originating within the LC, cell bodies of the majority of the noradrenergic neurons in the brain richly innervate numerous regions implicated in stress responses, including the extended amygdala, hippocampus, and prefrontal cortex, areas considered critical in mediating the alertness,

focus and many other cognitive and physiological changes necessary in dealing with stress (FOOTE and ASTON-JONES 1995; BUSCH et al. 1995). The volume of the rat LC and the number of NE neurons within the LC are larger in females than in male rats (GUILLAMON et al. 1988; LUQUE et al. 1992). The LC of females have a greater number of DBH immunoreactive cells. Sex differences in the rat LC are most evident at postnatal days 60-90 (PINOS et al. 2001). It was suggested that the LC differentiation depends on the organizational effects of estrogen during the neonatal period. Several experiments suggest that constant estradiol presence is necessary to maintain the feminine morphological pattern in the LC of the female rats.

Therefore we have been interested in the effects of estrogen on expression of CA biosynthetic enzymes as well as to the response to stress.

Administration of estradiol was found to modulate expression of TH, DBH as well as GTPCH genes both *in vivo* in and in cell culture (LIAW et al. 1992; ARBOGAST and HYDE 2000; SEROVA et al. 2002, 2006). The *in vivo* response depends on the mode of administration and the particular catecholaminergic tissue examined, and also likely the particular estrogen receptor subtype expressed (SEROVA et al. 2004; MAHARJAN et al. 2005). The studies in cell culture revealed that regulation is at the transcriptional level and that estradiol has a direct effect on TH, DBH and GTPCH promoter activity. Interestingly, in PC12 cells of adrenomedullary origin, estradiol elevated DBH and GTPCH promoter activity in the presence of ERα as well as ERß (SEROVA et al. 2002, 2006). However, TH responded in an opposite direction depending on the ER subtype expressed, estradiol triggered an elevation in the presence of ERα and a reduction with ERß (MAHARJAN et al. 2005).

Not only does estrogen regulate expression of genes related to CA biosynthesis, but it was found to alter the response of these genes to elevations in cAMP. Estradiol attenuated the cAMP mediated activation of TH promoter activity (ARBOGAST and HYDE 2000; MAHARJAN et al. 2005), while enhancing that of DBH and GTPCH (SEROVA et al. 2002, 2006).

To investigate the response to stress with and without estradiol, ovarectomized (OVX) female rats were pretreated for 16 days with estradiol benzoate (EB), or only the vehicle and then exposed to 2 hr IMO stress (SEROVA et al. 2005). Estradiol modulated many of the physiological responses to stress in OVX female rats. Several of the responses were attenuated, while some were actually opposite those of control animals. The

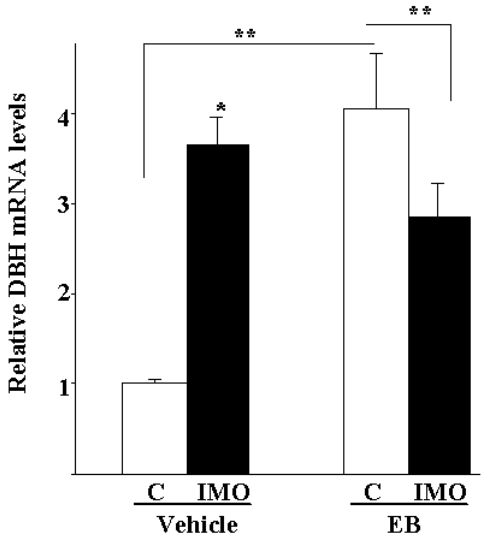

Fig 7 Estrogen modulates the response DBH to IMO stress. Ovariectomized female rats treated with estradiol benzoate (25 mg/kg) or vehicle (oil) (sc) once daily for 16 days were subjected to 2 hr IMO stress. Levels in LC were compared in unstressed controls (C) or after IMO [from (SEROVA et al. 2005)].

IMO stress triggered elevation in plasma ACTH levels which were lessened in EB pretreated animals. Similarly, there was no further change with stress in EB treated animals for TH mRNA and GTPCH levels in adrenal medulla. Interestingly, several responses were opposite in animals which had received injections of EB compared to the controls. Thus, IMO stress reduced DBH mRNA levels in the LC (Fig. 7), GTPCH mRNA and BH4 levels in the NTS, all of which were elevated by the same stress in the control animals. In addition, the prolonged effect of restraint on blood pressure was reduced in the EB treated rats.

We speculate that the interaction between estradiol and various signalling pathway, in particular the cAMP mediated pathway, may be crucial in alterations in the response to stress in estradiol treated animals, and may be very pertinent to sex differences in response of CA systems to stress.

Acknowledgements

The continued financial support of NIH is greatly acknowledged, particularly NS 28869 and NS44218. Other support for various aspects of this work were supported by grant from US Office of Naval Research,

US-Slovak Grants, Fogarty International Collaborative Award., and Wyeth Pharmaceuticals Inc. I gratefully acknowledge the gifted group of scientists, colleagues and collaborators, who contributed to this work and have made the research an enjoyable journey, especially to Dr. Lidia I. Serova. I dedicate this article to my close colleague and friend Dr. Richard Kvetnansky on the occasion of his 70th birthday.

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