Arch. Pharm. Res. Vol.19, No.5, pp. 374-380, 1996

The Effect of Chronic Ethanol Treatement and Cold Stress on Catecholaminergic Enzyme Activity and mRNA in Rat Brain and Adrenals

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(Received March 6, 1996)

Sprague-Dawley male rats (150 g) were chronically treated with 5 v/v % ethanol admixed with nutritionally complete liquid diet and fed ad libitum for 3 weeks. One half of each group was exposed to cold stress at 4°C either for 24 h (for determination of mRNA by in situ hybridization) or for 48 h (for determination of enzyme activity). Chronic ethanol treatment (ethanol) did not affect tyrosine hydroxylase(TH) mRNA level in locus coeruleus(LC) of brain and adrenal medulla(AM) compared to controls. Cold stress showed strong increase of TH mRNA level in LC and AM compared to controls. Pretreated ethanol reduced the increased TH mRNA level by cold stress in LC and AM. Ethanol did not affect TH activity in LC and adrenal glands(adrenals). Cold stress increased TH activity in LC but not in adrenals. Pretreated ethanol did not reduce the increased TH activity by cold stress in LC but this result was not shown in adrenals. Phenylethanolamine-N-methyltransferase(PNMT) activity in C_1C_2 and adrenals increased only in ethanol treated group. These results suggest that ethanol does not affect TH mRNA level and activity in LC and adrenals, but increases PNMT activity in C_1C_2 and adrenals in normal rat. It is also suggested that pretreated ethanol reduces the magnitude of cold stress response, that is induction of TH mRNA in LC and AM, and does not reduce the protein activation of TH that is also cold stress response in LC.

Key words : Ethanol, Cold stress, Tyrosine hydroxylase, Phenylethanolamine-N-methyltransferase

INTRODUCTION

Varying results have been obtained on the effect of chronic ethanol treatment(ethanol) on catecholamine levels in brain eversince Gursey and Olson (1960), and they are either a lack of effect or an elevation of catecholamine level according to the duration of treatment. But few reports have been obtainded on the effect of ethanol on catecohlaminergic enzyme system. Carlsson *et al.* (1973) reported that TH activity of whole brain was increased by a single dose of ethanol and Detering *et al.* (1980) reported that TH activity of whole brain was increased in offspring of rats fed a diet containing ethanol.

There are a few reports (Lehrer *et al.*, 1974; Levenson *et al.*, 1980; Sher *et al.*, 1982) that chronic ethanol consumption reduce the magnitude of response to stress in human or rat, occassionally that is said either tension reduction by lower doses of ethanol or stress response dampening by higher doses of ethanol. In those reports, ethanol reduced simple physiological or psychyatric responses to laboratory stressors such as loud tones, high affect words, shaking and electrical stimuli. Those responses are pulse transmission, cardic and electrothermal responses reported in 1980s. Biochemical respose change to stress was reported (Thoenen, 1970; Chuang *et al.*, 1974; Fluharty *et al.*, 1985; Tank *et al.*, 1985), that was TH activity increase by cold stress. TH mRNA increase by cold stress was also reported(Tank *et al.*, 1985; Stachowiak *et al.*, 1985; Stachowiak *et al.*, 1986).

In this study, we aimed to investigate ethanol effect on TH enzyme system either in normal rat or in cold stressed rat. According to the preliminary test in our laboratory, ethanol did not affect significantly TH activity in LC and adrenals (these data were not shown in this paper), therefore we investigated that how

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ethanol affect the cold stress responses. First, we investigated the effect of ethanol on TH activity and TH mRNA level in normal rat, and secondly we investigated the effect of ethanol on TH activity and mRNA level in acute cold stressed rat.

MATERIALS AND METHODS

Chronic Ethanol Treatment

Sprague-Dawley male rats (Charles River, Wilmington, MA) weighing 150 g were housed in individual shoe box cages bedded with shavings. One week after the arrival, the animals were paired according to the proximity of their body weights. One of each pair was assigned to the ethanol group and the other served as its pair-fed control. During the experiments, the sole source of diet and water was nutritionally complete liquid diet (Bio-serve, Liquid-Rat Diet, Lieber and DeCarli, 1982)

Ethanol was given *ad libitum*, admixed in liquid diet to make 5 v/v% final concentration. The control animals received the liquid diet containing sucrose that was made isocaloric to the 5 v/v% ethanol diet and pair-fed. The volume of liquid diet consumed and the body weight were measured and recorded daily. The amount of ethanol consumed was calculated and expressed as amount consumed per kg of body weight of the animal per day. The total duration of the treatment was 3 weeks.

Stress condition

One half of the ethanol treated group and its pair-fed animals were randomly selected and exposed to cold stress at 4°C either for 24 h (for determination of mRNA by in situ hybridization) or for 48 h (for determination of enzyme activity). The feeding of animals was continued as it is described as the above.

In situ hybridization

Rat were anesthetized with pentobarbital (50 mg/kg) intraperitoneally and then rapidly perfused transcardially with 0.9% sodium chloride, containing 0. 5% sodium nitrite and 1,000 U heparin/100 ml. This was followed by a slow perfusion with ice-cold 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. Brains and adrenals were immediately removed, cut into blocks, and submerged in the ice-cold fixative where they remained for 1 h. The blocks were then rinsed twice with phosphate buffer and cryoprotected by storing the tissue in 30% sucrose overnight at 4°C. Tissue sections of 30 um thickness were cut on a sliding mirotome and stored in 20 ml glass vials filled with 2xSSC (0.3M sodium chloride/0.03 M sodium citrate, pH 7.0) with 10 mM Dithiothreitol (DTT) at 4°C. Hybridization method was determined by a minor modification of the method of Young (1987). This storage solution was then replaced with prehybridization buffer containing 50% formaldehyde, 10% dextran sulfate, 2xSSC, 1xDenhart's solution, 50 mM DTT, and DNA. Prehybridization was carried out for 1 h at 48°C. As previously described, a 0.4 kb KpnI-EcoRI restriction fragment of rat TH cDNA, labeled with [^{35}S]dCTP by random priming, was added to each vial containing tissue sections as a probe. Hybridization was carried out overnight at 48°C. After extensive washes in decreasing concentrations of SSC, 10 min steps of 1:1 dilutions starting at 2xSSC and ending with 0.1xSSC, tissue sections were mounted onto gelatin-subbed slides, air-dried and dehydratede through graded ethanols (70, 90, 100%). Finally the slides were dipped into undiluted Kodak NTB-2 in the darkroom. After storage in light-tight boxes at 4°C for 7 to 21 days, the slides were developed in Kodak D-19, counterstained with cresyl violet and cover-slipped with Permount (Fisher Scientific).

Measurement of TH activity

The animal was briefly anesthetized by isoflurane and sacrificed by decapitation. LC of brain and adrenals were dissected bilaterally and homogenized each in 400 ul and 2 ml of 0.5 mM Tris-Cl buffer (pH 7.5) containing 0.1% Triton X-100, respectively. Homogenates were centrifuged at 10,000 g for 30 min. at 0°C. Supernatant was used for the measure - ment of TH activity. TH activity was measured by Waymire et al.(1971)'s method. The incubation mixture (total volume, 0.5 ml) contained (in umole): sodium acetate buffer (pH 6.1), 100.0; ferrous sulfate, 0. 5; 6.7-dimethyl-5,6,7,8-tetrahydropterine(DMPH$_4$), 1.0; mercapto ethanol, 20.0; sodium phosphate, 1.0; L-tyrosine-[1-^{14}C], 0.05 (10 uci/umole, 1.1×10 dpm); a crude kidney aromatic L-aminoacid decarboxylase prepration, 7.5 units (nmole/30 min); and pyridoxal phosphate, 0.005. The enzymatic reaction was carried out at 37°C for 30 min. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid. The radioactive CO_2 liberated from the L-DOPA-[1-^{14}C] formed from tyrosine-[1-^{14}C] by TH, was absorbed on a filter paper wetted with NCS solubilizer (Du Pont-New England Nuclear) and measured as described by Lamprecht and Coyle (1972) in the assay of dopa decarboxylase based on $^{14}CO_2$ evolution from [carboxy-^{14}C]-dopa. Specific activity is defined as pmole of dopamine formed/mg protein/min. Protein concentration was determined by the method of Lowry *et al.* (1951).

Measurement of PNMT activity

Phenylethanolamine-N-methyltransferase(PNMT) activity was determined by Axelord's method (1961) as

described elsewhere (Park, 1986). The incubation mixture contained o.5 mM phenylethanol-amine; 150 mM potassium phosphte buffer (pH 8.6); and enzyme solution containing 10 ug bovine serume albumine in a final volume of 100 ul. The extration procedure for the reaction product was same as those of DBH (Molinoff et al. 1971). Specific activity of PNMT is defined as pmole of N-methyl-phenylethanolamine formed/mg protein/15 min at 37°C.

Statistical analysis

Statistical analysis of the data was performed using student's t-test compared to control rat. Only the ethanol plus cold stressed group was analyzed by two way ANOVA test between cold stress and ethanol treatment.

RESULTS

Chronic ethanol treatment

The amount of ethanol consumed was calculated and expressed as amount consumed per kg of body weight of the animal per day in Fig. 1.

TH mRNA

Fig. 2. shows that cold stress leaded to strong induction of TH mRNA in LC but pretreated ethanol appeared to reduce the increased TH mRNA by cold stress in LC. In contrast, ethanol alone did not show any change in TH mRNA level.

The changes of TH mRNA level resulting from ethanol and cold stress in adrenal medulla are shown in Fig. 3. Ethanol alone did not cause any alteration in message levels, but cold stress increased TH mRNA level similar to the observation made in LC. Pretreated ethanol also reduced the increased TH mRNA by cold stress in AM like the LC.

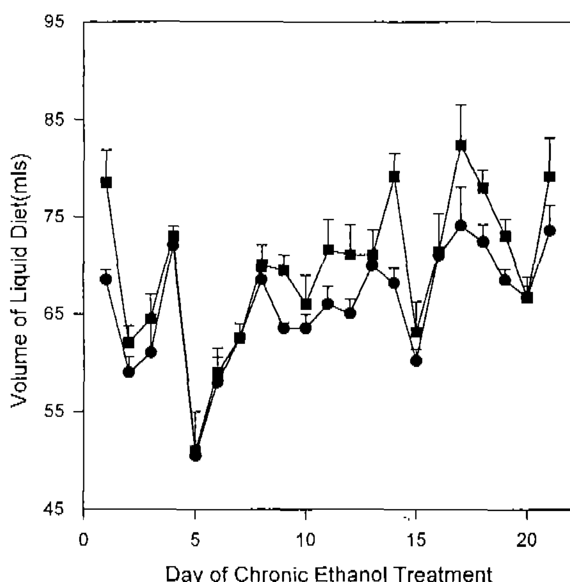

Fig. 1. Daily Consumption of Liquid Diet Pair-fed Control (●—●) vs Ethanol (■—■)

Enzyme activity

Table 1. summarizes the effect of ethanol on combination with acute cold stress on TH activity of rat LC and adrenals. TH activities of ethanol group in LC and adrenals were not changed significantly compared to control group. TH activity of cold stressed group significantly increased compared to control group only in LC. TH activity of ethanol plus cold stressed group significantly increased compared to cold stressed group and compared to ethanol group, but this increasement was not shown in adrenals.

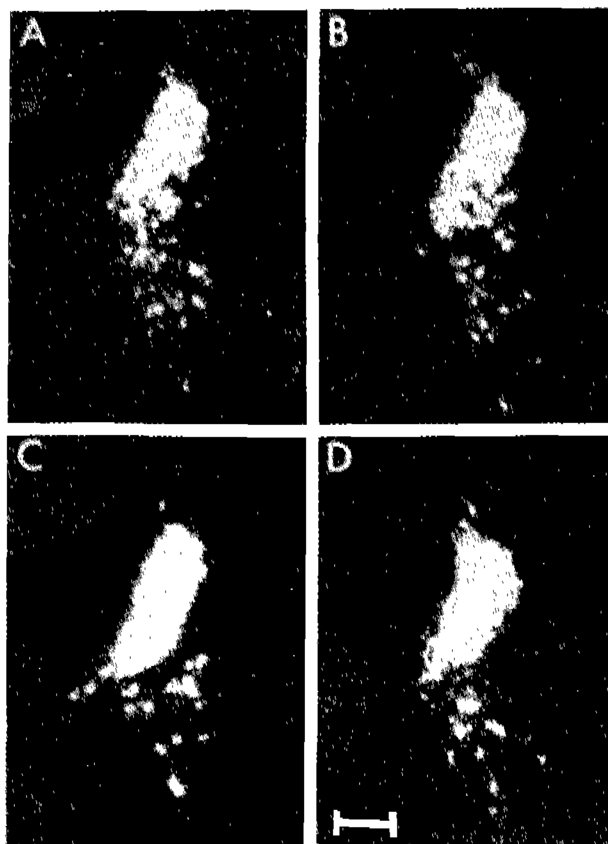

Fig. 2. *In situ* hybridization to THmRNA in LC. Dark-field photomicrographs illustrate hybridization signals for TH message of isocaloric sucrose fed control animals (A), those chronically treated with ethanol (B), those acutely exposed to cold (C), and those chronically treated with ethanol followed by acute exposure to cold (D). Note that cold stress leads to strong induction of TH mRNA in LC but ethanol treatment appears to reduce the increased TH mRNA levels by cold stress in LC. In contrast, ethanol tretment alone do not show any change in grain density. Bar=200 um

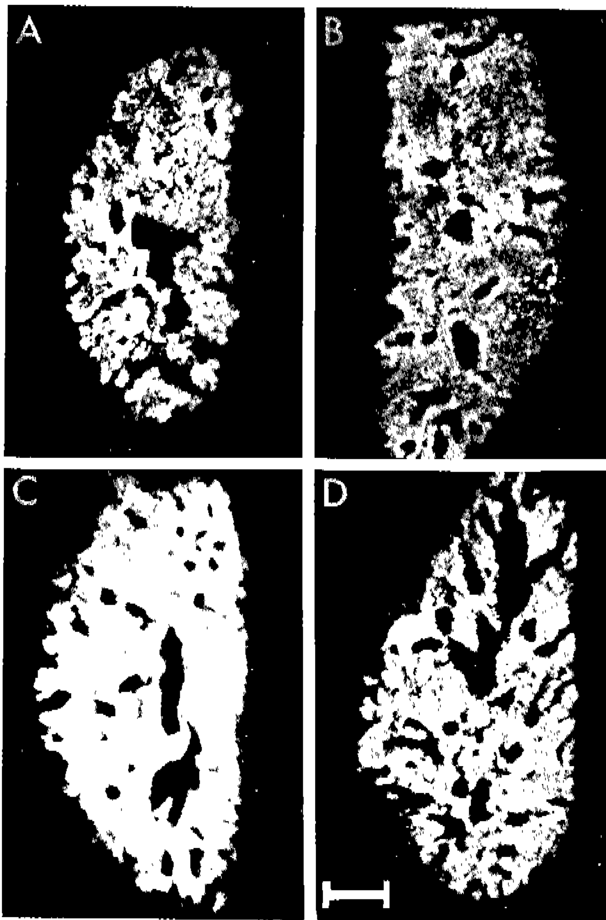

Fig. 3. In situ hybridization to TH mRNA in adrenal medulla (AM) of the rat. Dark-field photographs illustrate hybridization signals for TH message of isocaloric sucrose fed control animals (A), those chronically treated with ethanol (B), those acutely exposed to cold (C), and those chronically treated with ethanol followed by acute exposure to cold (D). Note that ethanol treatment alone do not cause any alteration in message levels, but cold stress also induces strong response in the message levels similar to the observation made in LC. Ethanol also reduce the increased TH mRNA levels in AM like the LC. Bar=300 um

Table 2 summarizes the effect of ethanol on combination with acute cold stress on PNMT activity of rat C_1C_2 and adrenals. PNMT activities of ethanol group in C_1C_2 and adrenals significantly increased compared to control group. PNMT activities of cold stressed group in C_1C_2 and adrenals were not changed. PNMT activities of ethanol plus cold stressed group did not show difference significantly compared to controls and cold stressed group in C_1C_2 and adrenals, but showed interactions between cold stress and ethanol.

DISCUSSION

In this work, we tested ethanol effect on TH and PNMT in LC and adrenals in normal and cold stressed rat. LC is a compact catecholaminergic nucleus (Dahlstrom *et al.*, 1964; Ungerstedt *et al.*, 1971; Astone-Jone *et al.*, 1984). C_1C_2 is PNMT containing cell bodies in brain (Hokfelt *et al.*, 1973; 1974; Lew *et al.*, 1977) and adrenals have catecholaminergic neurons pheripherally.

Table I. Differential Effects of Ethanol in Combination with Acute Cold Stress on Tyrosine Hydroxylase (TH) Activity of Rat Locus Coeruleus and Adrenals

Treatment	Tyrosine Hydroxylase Activity	
	Locus Coeruleus	Adrenal Glands
Control	0.164±0.0187 (5)	6.69±0.279 (5)
Ethanol	0.152±0.0128 (5)	6.40±0.145 (5)
Cold Stress	0.253±0.0289* (5)	6.84±0.273 (5)
Ethanol+Cold stress	0.394±0.0467◆ (5)	6.57±0.816 (5)

TH activity (mean±SEM) is expressed in nmol/mg protein/15 min 30°C. Number of animals used is given in parenthesis.
*p<0.05 when compared to control
◆p<0.05 when compared to ethanol and cold stress by two way ANOVA test

Table II. Differential Effects of ethanol in Combination with Acute Cold stress on PNMT Activity of Rat C_1C_2 and Adrenal Glands

Treatment	PNMT	
	C_1C_2	Adrenal Glands
Control	15.947±0.837 (5)	793.4±66.6 (5)
Ethanol	18.366±0.620** (5)	1007.0±35.51** (5)
Cold Stress	15.120±0.640 (5)	726.5±45.8 (5)
Ethanol+Cold stress	14.181±0.469◆ (5)	679.3±41.3 (5)

PNMT activity (mean±SEM) is expressed in pmol/mg protein/15 min. at 37°C. Number of animals used is given in parentheses.
**p<0.01 when compared to control
◆p<0.05 when compared to ethanol and cold stress by two way ANOVA test

Fig. 1 and Fig. 2 show that ethanol did not change TH mRNA level in LC and AM and cold stress leaded to strong induction of TH mRNA in both LC and AM of normal rat, but ethanol reduced the increased TH mRNA level by cold stress in both LC and AM. Our result that one day cold stress leaded to strong induction of TH mRNA in LC and AM agrees with the other results (Tank *et al.*, 1985). It is considered that ethanol alone do not affect TH mRNA level in normal rat, but pretreated ethanol supress the cold stress response that is strong induction of TH mRNA in both LC and AM.

From the reports (Tank *et al.* 1985; Stachowiak *et al.* 1985; Stachowiak *et al.* 1986) that enzyme activity increase is preceded by mRNATH level increase, we investigated the TH activity both in LC and adrenals. From the result shown in Table I, it is suggested that ethanol does not affect TH activity

both in LC and adrenals. It is considered that TH activity change was not shown because of being not preceeded by THmRNA change by ethanol (shown in Fig. 2 and Fig. 3).

From the results shown in Fig. 2 and Table I, TH activity of ethanol plus cold stressed group was increased about 50% more than that of cold stressed group in LC (Table I), though TH mRNA level was decreased compared to cold stressed group (Fig. 2). It is considered that the reason of this discrepancy between TH mRNA level and TH activity of ethanol plus cold stressed group may be due to a duration of cold stress. We measured mRNA level after one day cold stress and enzyme activity after two days cold stress, each stress are acute. It is also considered that two days cold stress can be in the middle of maximum increasement of TH mRNA and TH activity by cold stress. Fluharty *et al.* (1985) get a maximal TH activity increase about 4 fold in adrenals by a chronic cold stress for more than 6 days. It is also asked the development of the experimental method that enzyme activity and mRNA level can be measured at the same time in one small sample like as LC.

From the result shown in Table II, ethanol increased PNMT activity in C_1C_2 and adrenals compared to control, this result agrees with Pohorecky *et al.* (1974), who reported that ethanol increased PNMT activity 16 % in adrenals. Compared to the data shown in Table I, ethanol did not affect TH activity in LC and adrenals. It is considered that disconcordant action of ethanol in TH and PNMT activity may be mediation of adrenal cortical hormon by ethanol only in PNMT activity. Wurtman (1966) and Hasselbach *et al.* (1982) reported that adrenal PNMT activity increase was mediated by adrenal hormon concentration, and reported that adrenal cortical hormon concentration was increased by ethanol(Gordon and Southern, 1980; Knych and Prohaska, 1981; Wright, 1978). Cold stress did not change PNMT activity significantly, this result agrees with Kvetnansky *et al.* (1971) who reported that less than 7 days cold expossure did not show any change in PNMT activity. More investigation to estimate alterations in the expression level of PNMT gene. From the result that ethanol plus cold stressed group showed interactions between ethanol group and cold stressed group, it is suggested that cold stress can supress the ethanol effect that increased PNMT activity in normal rat.

Immunochemical studies demonstrated that the increased TH activity reflected an enzyme protein increase (Hoeldtke *et al.*, 1974) and suggested that adrenal TH activity increase was mediated by an concomitant increase in intracellular enzyme protein concentration (Joh *et al.*, 1973; Reis *et al.*, 1974; Tank *et al.* 1985). From those suggestions, it is hypothesized that ethanol pretreatment do not supress the increased TH enzyme protein amount by cold stress in LC from the result that ethanol pretreatment did not reduce the increased TH activity by cold stress in LC. It is also hypothesized that ethanol increased PNMT protein level in C_1C_2 and adrenals.

From the results shown in Fig. 2, Fig. 3 and Table I, cold stress incresed TH mRNA level and TH activity in LC, but cold stress incresed only TH mRNA level without accompanying with TH activity increase in adrenals. From this discordant results in TH mRNA and activity between LC and adrenals, it is considered that LC can respond more sensitively than adrenals to two days cold stress because LC is a more compact nucleus consisting almost entirly of the cell bodies of catecholaminergic neurons than adrenals (Fuxe *et al.*, 1978; Iacovitti *et al.*, 1981; Biguet *et al.*, 1986).

In conclusion, the results from this study indicate that ethanol does not affect the transcription of the gene for TH both in LC and AM and subsequently does not affect the TH enzyme protein level in normal rat, but ethanol increases PNMT enzyme protein level. It is also indicated that pretreated ethanol reduces the increased transcription level for TH by acute cold stress both in LC and AM, and does not reduces the increased protein level for TH by cold stress in LC. More investigation about the effect of pretreated ethanol on TH enzyme protein is needed with chronic cold stress.

ACKNOWLEDGEMENT

This project has been partially supported by the research grant, AA-07724 from NIAAA.

RERENCES CITED

Aston-Jones, G., Foote, J. L. and Bloom, F. E., Anatomy and physiology of locus coeruleus neurons: Functional implications, Ziegler, M. G. and Lake, C.R.(eds.) Norepinephrine. Baltimore, Williams and wilkins, 1984, sction 3, pp 92-100.

Axelord, J., Purification and properties of phenylethanolamime-N-methyltransferase. *J. Biol. Chem.*, 237, 1657-1660 (1961).

Biguet, N. F., Buda, M., Lamouroux, A., Samolyk, D. and Mallet, J., Time course of the changes of TH mRNA in rat brain and adrenal medulla after a single injection of reserpine. *EMBO J.*, 5(2), 287-291 (1986).

Calsson A. and Margit, L., Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain *in vivo*. *J. Pharm. Pharmac.*, 25, 437-440 (1973).

Chuang, D. and Costa, E., Biosynthesis of tyrosine hydroxylase in rat adrenal medulla after exposure to cold. *Proc. Nat. Acad. Sci.*, 71, 4570-4574 (1974).

Dahlstrom, A. and Fuxe. K., Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta. Physiol. Scand.*, 62(Suppl 232), 1-55 (1964).

Detering, N., Edwards. E., Ozand P. and Karahasan, A., Comparatitve effects of ethanol and malnutrition on the development of catecholamine neurons: changes in specific activities of enzyme. *J. Neurochem.*, 34(2), 297-304 (1980).

Fluharty, S. J., Snyder, G. L., Zigmond, M. J. and Stricker, E. M., Tyrosine hydroxylase activity and catecholamine biosynthesis in the drenal medulla of rats during stress. *J. Pharmacol. Exp. Ther.*, 233, 32-38 (1985).

Fuxe, K., Hokfelt, T., Agnati, L.F., Johannson, O., Goldstein, M., Perez, M., Possani, L., Tapia, R., Teran, L. and Palacios, R. (1978) in Psychophamacology (Lipton, M. A., DiMascio, A., and Killam, K. F., eds). pp. 67-110, Raven Press, New York.

Gordon, G. G. and Southern, A. L., The effect of alcohol and alcoholic liver disease on the biosynthesis of steroid in rat reticular homogenates *in vitro. Endocrinology*, 106, 1980-85 (1985).

Gordon, G. G. and Southern, A. L., The effect of alcohol and alcoholic liver disease on the biosynthesis of steroid in rat reticular homogenates *in vitro. Endocrinology*, 106, 1980-1985 (1985).

Gursey, D. and Olson, R. E., Depression of serotonine and norepinephrine levels in brain stem of rabbit by ethanol. *Proc. Soc. Exp. Biol. Med.* 104, 280 (1960).

Hasselbach, H., Selmer, L, Sestoft, L. and Kehlet, H., Hypothalamic-pituitary-adrenocortical function in chronic alcoholism. *Clin. Endocrinology*, 16, 73-76 (1982).

Hokfelt, T., Fuxe, K., Goldstein, M and Johansson, O., Evidence for adrenaline neurons in the rat brain. *Acta. Physiol. Scand.*, 89, 286-288 (1973).

Hokfelt, T., Fuxe, K., Goldstein, M and Johansson, O., Immunohistochemical evidence for the existance of adrenaline neurons in the rat brain. *Brain Res.*, 66, 235-251 (1974).

Hoeldtke, R., Lloyd, T. and Kaufman, S., An immunochemical study of the induction of tyrosine hydroxylase in rat adrenal glands. *Biochem. Biophysics. Res. Commun.*, 57, 1045-1053 (1974).

Iacovitti, L., Reis, D. J. and Joh, T. H., Reactive proliferation of brainstem noradrenergic nerves following neonatal cerebellectomy in rats: role of target maturation on neuronal response to injury during development. Develop. brain. Res., 1, 3-24 (1981).

Joh, T. H., Geghman, C. and Reis, D. J., Immunochemical demonstration of increased accumulation of tyrosine hydroxylase protein in sympathetic gangria and adrenal medulla elicitetd by reserpine. *Proc. Nat. Acad. Sci.*, 70, 2667-2771 (1973).

Knych, E. T. and Prohaska, J. R., Effect of chronic intoxication and naloxone on ethanol-induced increase in plasma corticosterone. *Life Sci.*, 28, 1987-1994 (1981).

Kvetnansky, R., Gerwitz, G.P., Weise, V.K. and Kopin, I.J., Catecholamine synthesizing enzymes in the rat adrenal gland during exposure to cold. *Am. J. Physiol.*, 220, 928-931 (1971).

Lamprecht, F and Coyle, J. T., DOPA decarboxylase in the developing rat brain. *Brain Res.*, 41, 503-506 (1972).

Lehrer, P. M. and Taylor, H. A., Effect of alcohol on cardiac reactivity in alcohols and nonalcoholics. *Quarterly J. Studies on alcohol*, 35, 1044-1052 (1975).

Levenson, R. W., Sher, K. J., Grossman, L. M., Newman, J. and Newlin, D. B., Alcohol and stress dampening: pharmacological effects, expectancy, and tension reduction. *J. Abnorm. Psychol.*, 91, 350-368 (1982).

Lew, J. Y., Matsumoto, Y., Pearson, J., Goldstein, M., Hokfelt, T. and Fuxe, K., Localization and characterization of phenylethanolamine N-methyl transferase in the brain of various mammalian species. *Brain Res.*, 119, 199-210 (1977).

Lieber C. S. and De Carli L. M., An experimental model of alcohol feeding and liver injury in the baboon. *J. Med. Prim.*, 3, 153-163 (1982).

Lowry, O. H., Rosenburg, N. J., Farr, A. L. and Randall, R. J., Protein measuremint with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Molinoff, P. B., Weinshilboum, R. and Axelord, J., A sensitive enzymatic assay for dopamine-B-hydroxylase. *J. Pharm. Exp. Ther.*, 178(3), 425-431 (1971)

Park, D. H., Monoamine methyltransferase: phenylethanolamine-N-methyltransferase and catecholamine-O-methyltransferase, In A.A. Boulton, G.B. Baker and P.H. Yu (Eds.), Neuromethods. Vol. 5. Neurotransmitter Enzymes, Humana Press, Clifton, NJ, 1986, pp. 117-145.

Pohorecky, L. A., Jaffe, L. S. and Bekery, H. A., Effects of ethanol on the adrenal medulla of the rat. *Pharmacology*, 12, 340-346 (1974).

Reis, D. J., Joh, T. H., Ross, R. A. and Pickel, V. M., Reserpine selectively increase tyrosine hydroxylase and dopamine-B-hydroxylase enzyme protein in central noradrenergic neurons. *Brain Res.*, 81, 380-386, 1974.

Sher, K.J., Risk for alcoholism and individual diff-

erences in the stress-response-dampening effect of alcohol. *J. Abnorm. Psychol.*, 91, 350-367 (1982).

Stachowiak, M. K., Sebbane, R., Stricker, E. M., Zigmond, M. J. and Kaplan, B. B., Effect of chronic exposure on tyrosine hydroxylase mRNA in rat adrenal gland. *Brain Res.*, 359, 356-359 (1985).

Stachowiak, M.K., Fluharty, S. J., Stricker, E. M., Zigmond, M. J. and Kaplan, B. B., Molecular adaptations in catecholamine biosynthesis induced by cold stress and sympathectomy. *J. Neurosci. Res.*, 16, 13-24 (1986).

Tank, A. W., Lewis, E. J., Chikaraishi, D. M. and Weiner, M., Elevation of RNA coding fir tyrosine hydroxylase in rat adrenal gland by reserpine treatment and exposure to rat. *J. Neurochem.*, 45, 1030-1033 (1985).

Thoenen, H., Induction of tyrosine hydroxylase in peripheral and central adrenergic neurons by cold exposure of rats. *Nature*, 228, 861-862 (1970).

Ungerstedt, U., Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta. Physiol. Scand.*, 82(Suppl), 367, 1-48 (1971).

Young, W.S.(1987) in Application to Neurobiology. (Valentino, K. L. Eberwine, J. H. and Barchas, J. D. eds) Oxford University Press, pp 27-40, New York, N.Y.

Waymire, J. C., Bjur. R. and Weiner, N., Assay of tyrosine hydroxylase by coupled decarboxylation of dopa formed 1-^{14}C-L-Tyrosine. *Anal. Biochem.*, 43, 588-600 (1971).

Wright, J., Endocrine effects of alcohol. Clin. Endocrinol. Metab., 7, 351-367 (1978).

Wurtmann, R. J., Control of epinephrine synthesis in the adrenal medulla by the adrenal cortex; hormonal specificity and dose-response characteristics. *Science*, 188, 1085-1089 (1966).