with hydralazine and dialyzed against the buffer under the same conditions mentioned above. Only about 8 percent inhibition of lysyl oxidase activity was observed with the hydralazine-treated substrate. These results suggest that irreversible hydralazine action is mainly directed at the enzyme.

Prolyl hydroxylase activity was restored maximally (about 80 percent) by the addition of Fe^{2+} to the incubation mixtures containing hydralazine because of its chelating reaction with Fe^{2+} [5]. The hydralazine-induced inhibition of dopamine β-hydroxylase activity was almost reversed by the addition of Cu^{2+} which is essential for the inactivation of the endogenous inhibitors present in the enzyme preparations [6]. The inhibitory effect of hydralazine on the collagen secretion was also reversed by Fe^{2+} alone or Fe^{2+} together with Mn^{2+} [7].

However, hydralazine-induced inhibition of lysyl oxidase activity could not be reversed even by the intensive dialysis of inhibited enzyme preparation against 0.05 M potassium phosphate buffer containing 0.1 mM $CuCl_2$.

It is now almost established that a pyridoxine derivative [8, 9], probably pyridoxal-phosphate, is another essential cofactor of lysyl oxidase, in addition to copper ion [10–12]. Since lysyl oxidase is inhibited by some carbonyl reagents [11], hydralazine might react with pyridoxal cofactor of lysyl oxidase. We failed, however, to restore the activity of inhibited enzyme by dialyzing intensively against the phosphate buffer containing 0.05 mM pyridoxal-phosphate.

Since hydralazine combined with proteins as hydralazones [13] and with sulfhydryl compound [14], hydralazine might combine directly with the protein moiety of lysyl oxidase. As for the sulfhydryl group, it has been demonstrated that chick cartilage lysyl oxidase contains a sufficient number (30/1000 residues) of half cysteines which could contribute to the stability of the enzyme molecules [15].

Further investigations on the mechanism of hydralazine inhibition are under way along these lines in our laboratory.

In summary, lysyl oxidase activity was inhibited by hydralazine *in vitro* (50 percent inhibition at about 30 μM). Hydralazine seems to bind to the enzyme fairly tightly, because the inhibition was not reversed by intensive dialysis against phosphate buffer. The activity could not be restored even by the dialysis of inhibited enzyme against copper or pyridoxal-phosphate solution.

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Cyclic AMP response to norepinephrine in the limbic forebrain of male and female rats: effect of desipramine

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Cytoplasmic receptors exist in certain brain areas for steroid hormones [1] which could act directly or indirectly as endocrine regulators of neuronal activity. Through the combined use of autoradiography and fluorescence histochemistry, Heritage *et al.* [2] have identified estradiol target sites in nuclei of the major norepinephrine (NE) containing cell bodies in the brain stem, thus providing a neuroanatomical basis for physiological interactions between sex steroid hormones and NE in brain. Moreover, chronic exposure of ovariectomized rats to 17α-ethynylestradiol has been reported to decrease beta adrenergic cyclic AMP responses in the cortex linked to a reduction in the density of beta adrenergic membrane receptors [3]. Since beta adrenergic receptors in brain represent a subpopulation of noradrenergic receptors coupled to adenylate cyclase [4, 5] and since most if not all antidepressant treatments down-regulate the NE receptor coupled adenylate cyclase system in brain [6], it was of interest to determine whether

or not sex differences exist in the responsiveness of the cyclic AMP generating system to NE and/or the change in noradrenergic sensitivity following chronic treatment with a tricyclic antidepressant.

Male and female Sprague–Dawley rats (180–200 g) were used. Standard laboratory diet (Purina Food Co.) and water were provided *ad lib*. The animals were maintained under standard laboratory conditions with a controlled 12 hr light–dark cycle. Desipramine (DMI) was administered intraperitoneally (15 mg · kg^{-1} · day^{-1}) for a period of 7 days. The animals were decapitated 24 hr following the last injection with the drug, the brains were quickly removed, and the area referred to as the limbic forebrain area was dissected as described previously [7]. The NE-induced cyclic AMP responses were determined in tissue slices according to methods previously described [4, 7]. Cyclic AMP was isolated by ion exchange chromatography and assayed by the method of Gilman [8]. Protein was determined in 0.5 ml aliquots of the tissue homogenate according to Lowry *et al.* [9]. The significance of the data was evaluated by Student's two-tailed *t*-test.

While there was no difference in the basal levels of cyclic AMP between limbic forebrains from male or female rats, the response of the cyclic AMP generating system to 100 μM NE was significantly lower in brain tissue from female rats compared to that in brain tissue from male animals (Fig. 1). Recently, Wagner and Davies [10] reported that the ability of isoproterenol to stimulate cyclic AMP in cortical slices was reduced in cycling female rats relative to male rats of the same age. Such data are in accord with the findings indicating that beta adrenergic receptors represent a subpopulation of NE receptors coupled to adenylate cyclase [4, 5].

The next experiment was undertaken to ascertain whether or not sex-related differences exist in the induction of noradrenergic subsensitivity in the limbic forebrain. Thus, DMI was administered daily in a dose of 15 mg/kg, i.p., for a period of 7 days. This treatment reduced the responsiveness of the cyclic AMP generating system to 100 μM NE in both sexes by approximately 50 percent (Table 1).

Estrogens have been shown to increase the turnover and/or release of catecholamines [11, 12] which, in turn, could lead to agonist-induced down-regulation of the NE

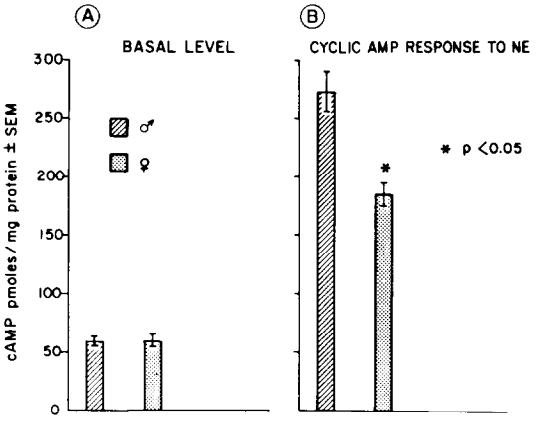

Fig. 1. Responsiveness of the cyclic AMP generating system to norepinephrine in the limbic forebrain of male and female rats. Tissue slices were incubated for 10 min in the absence or presence of 100 μM norepinephrine (NE), and cyclic AMP was isolated and determined as described in the text. The cyclic AMP response represents the NE-stimulated level minus the basal level of the nucleotide. Vertical bars represent the standard error of the mean. The number of animals used in each group was nine. Samples from each animal were assayed in duplicate.

Table 1. Down-regulation by desipramine of the noradrenergic cyclic AMP generating system*

	Response to 100 μM NE	
	Controls	DMI
Males	274 ± 36 (9)	127 ± 13† (10
Females	184 ± 18‡ (10)	110 ± 22§ (10)

* Animals were treated daily with desipramine (DMI, 15 mg/kg, i.p.) or saline (controls) for a period of 7 days. The response designates the NE-stimulated level of cyclic AMP minus the basal level of the nucleotide. Basal levels of cyclic AMP/mg protein ± S.E.M. were: males, 58 ± 6 (10) for controls and 51 ± 4 (10) for DMI-treated; females, 51 ± 4 (10) for controls and 58 ± 4 (10) for DMI-treated. Numbers in parentheses indicate the numbers of animals. Samples from each animal were determined in duplicate.

† $P < 0.005$ (male control vs male DMI).

‡ $P < 0.05$ (male vs female control).

§ $P < 0.025$ (female control vs female DMI).

receptor coupled adenylate cyclase system and could thus explain the observed reduced responsiveness to NE. Alternatively, estrogens could affect, via translocation of the cytoplasmic estrogen receptor complex to the nucleus, the synthesis of proteins involved in receptor-mediated cyclic AMP responses (e.g. NE receptors, nucleotide regulatory proteins). The reported slow development of changes in the density of beta adrenergic receptors following ovariectomy [10] would be consistent with such a mechanism. The down-regulation of the NE receptor coupled adenylate cyclase system by DMI has been shown to be dependent on intact presynaptic nerve terminals as both the subsensitivity to NE and/or isoproterenol and the reduction in the density of beta adrenergic receptors are prevented in animals pretreated with 6-hydroxydopamine [13, 14]. Since the DMI-induced subsensitivity of the NE receptor coupled adenylate cyclase system was quantitatively equal in both sexes, an interaction of ovarian steroids with the receptor occupancy by NE seems unlikely.

Although studies with adult castrates have not been performed, the observed equal down-regulation of the NE receptor coupled adenylate cyclase system by DMI in both female and male rats is compatible with recent findings on hormone–drug interaction and beta adrenergic receptor binding. Thus, while ovariectomy abolished the imipramine-induced decrease in $5HT_2$ receptor binding in rat brain cerebral cortex, the decrease in beta adrenergic receptor binding following long-term administration of imipramine was unaltered by ovariectomy [15]. It remains to be seen, however, whether the DMI-induced subsensitivity to NE develops faster in female than in male rats.

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Conversion of alpha-ketobutyrate to alpha-amino-*n*-butyric acid by isolated rat liver cells: effect of ethanol

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Chronic alcohol consumption results in increased plasma alpha-amino-*n*-butyric acid (AANB) in man [1] as well as in experimental animals (rats and baboons) [2]. According to Shaw and Lieber [3, 4], this rise is due, at least in part, to excess hepatic production possibly associated with decreased peripheral utilisation. The mechanism of increased net hepatic production of AANB is unknown. The breakdown of methionine being increased [5] and citric cycle activity depressed [6] during chronic alcohol intoxication, a decreased oxidation and an increased availability of a α-ketobutyrate (α-KB) (the precursor of AANB) may be postulated. However, a possible effect of ethanol on the conversion of α-KB to AANB could also take place. We used therefore freshly isolated hepatocytes to define the metabolic pathway involved in the conversion of α-ketobutyrate into AANB and the effects of ethanol on this conversion.

Materials and methods

Isolation and incubation of cells. Hepatocytes were isolated according to Krebs *et al.* [7] from 18 hr fasted female Sprague–Dawley rats of approx 200 g weight. The cells were incubated at a final concentration of 2×10^6 cells/ml in Krebs–Ringer bicarbonate buffer at pH 7.4 and 37°. Incubations were carried out for 60 min in a Metabolyte gyrotatory shaker (New Brunswick) at 90 oscillations/min under 95% O_2 + 5% CO_2. After storage on ice up to use, hepatocytes were first preincubated for 10 min in the presence or absence of 2 mM α-KB and either 4 mM L-glutamine or L-asparagine or 2 mM ammonium chloride. When indicated, 2 mM aminooxyacetate was added.

Sample preparation and amino acid analysis. For amino acid analysis, the samples were deproteinised with sulphosalicylic acid (4% w/v, final concentration). After centrifugation at 4° during 10 min at 3000 *g*, the clear supernatant was collected and adjusted to pH 2.2 with lithium hydroxide (10%, w/v). Free amino acids in the cell extracts (kept at −90° until analysed) were measured with an automatic amino acid analyser (Liquimat, Labotron, West Germany) [8].

Ethanol determinations. Ethanol (final concentration 10 mM) was added after the preincubation period. The reaction was stopped 60 min later by adding ice-cold perchloric acid (3% w/v, final concentration) and the supernatant used for ethanol determination according to Bernt and Gutman [9].

Chemicals. Collagenase (grade II), other enzymes and coenzymes were from Boehringer, Mannheim, West Germany; α-KB (sodium salt), L-glutamine and L-asparagine. H_2O from Sigma Chemical Co., St. Louis, MO. The other chemicals used were of analytical grade.

Expression of results. The results are expressed as μmoles of substrate removed or produced per gram wet weight of liver (on the basis of 10^8 cells per g wet weight of liver) and per time unit. The results are given as the values from at least two representative experiments from different liver cell preparations.

Results and discussion

Cell viability. All preparations of cells satisfied the following criteria: (a) the ATP content was 20–25 nmoles/10^6 cells; (b) the lactate dehydrogenase activity in the supernatant was less than 10% of the total activity; (c) the rate of ethanol oxidation during incubation with 10 mM ethanol (at which concentration ethanol is mainly metabolised by alcohol dehydrogenase reaction [10]) was about 0.9 μmoles/min/g liver wet weight as reported by several authors for similar preparations [10–12].

Formation of alpha amino-n-butyric acid (AANB) from α-ketobutyrate (α-KB) in isolated rat liver cells. As shown in Table 1, isolated rat liver cells were able to synthesise about 1.60 μmoles of AANB per g liver wet wt when