Table 1. Concentrations of tyrosine and total tryptophan in serum during the induction of ethanol dependence and during the withdrawal syndrome

Treatment	Tyrosine μg/ml	Tryptophan μg/ml
Control	16.4 ± 1.64	18.5 ± 2.00
Ethanol 5 days	17.6 ± 1.38	13.2 ± 1.1*
Ethanol 10 days	24.9 ± 2.83*	29.1 ± 4.81*
Withdrawal 5 h	34.1 ± 1.18†	21.9 ± 2.53
Withdrawal 10 h	25.6 ± 0.86*	20.8 ± 2.53

This Table shows the concentrations of tyrosine and total tryptophan found in the serum of mice during the inhalation of ethanol or during its withdrawal (further details see text). Each value represents the mean S.E. of at least five determinations. Asterisks indicate a difference from control values significant at the $P < 0.05$ level* or the $P < 0.01$ level† in the Student's "t" test.

centrations of total tryptophan in serum were elevated, but this was not significant at any time studied. These results are shown in Table 1.

DISCUSSION

Results show that alterations in peripheral metabolism of amino acids may contribute to changes in central neurotransmitter metabolism associated with the induction of ethanol dependence. Increases in catecholamine concentrations [1] and synthesis [2], in brains of animals rendered ethanol-dependent, or undergoing the ethanol withdrawal syndrome, may be related to the increases in serum tyrosine concentration reported here. The situation is more complex with regard to tryptophan metabolism, but it seems that the small increase in free tryptophan observed in ethanol-dependent animals could contribute to alterations in 5-hydroxytryptamine metabolism in the brain [1, 2]. Certainly changes in peripheral handling of endogenous, or administered, amino acid precursors must be considered as contributory factors in assessing results on central monoamine metabolism obtained during chronic administration of ethanol. This factor may explain some discrepancies in the literature [3, 13].

The reasons for the changes in serum amino acid concentrations reported here remain obscure. It seems likely that hepatic metabolism of amino acids plays a role [7, 8]. The non-specific stress of ethanol administration and withdrawal could also be important.

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Possible mechanism of action of propranolol in hypertension

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In recent years, propranolol, a β-adrenoceptor-blocking drug, has been used in the management of essential hypertension. The mechanism by which this drug lowers blood pressure is still speculative. After intravenous administration of propranolol, cardiac output decreases in all hypertensive patients but arterial pressure remains unaltered. Chronic therapy with the drug is needed to lower the blood pressure. A fall in blood pressure is associated with a decrease in the initially elevated peripheral resistance. A big advantage of propranolol is that lying and standing pressures are equally lowered and there is no orthostatic hypotension.

Among the factors that may be involved in propranolol-induced lowering of blood pressure are: (1) decreased cardiac output, (2) inhibition of renin release, and (3) diminution of tonic sympathetic nerve outflow from the vasomotor center in the brain. However, all these factors do not explain the long time lag between the administration of propranolol and the fall in blood pressure.

The present study shows that prolonged treatment with propranolol causes a reduction in adrenal tyrosine hydroxylase (TH) activity of the spontaneously hypertensive rat (SHR), an animal model that closely resembles human essential hypertension [1, 2]. Since TH activity is related to impulse traffic in a sympathetic nerve, a reduction in TH activity would indicate a decrease in sympathetic nerve

Table 1. Effects of propranolol on systolic blood pressure and tyrosine hydroxylase (TH) activity in the adrenal glands of the spontaneously hypertensive rat*

Treatment	Days of treatment	Systolic blood pressure (mm Hg)	Adrenal activity
Saline	SHR control	192 ± 2.4 (18)	100 ± 5.2 (18)
(−)-Propranolol	4	190 ± 3.6 (6)	98 ± 4.2 (6)
	8	158 ± 2.9† (6)	78 ± 2.2† (6)
	12	142 ± 1.6‡ (6)	64 ± 3.6‡ (6)
(+)-Propranolol	12	192 ± 2.6 (6)	97 ± 6.0 (6)

* Spontaneously hypertensive rats (SHR) were given (−)-propranolol or (+)-propranolol (2 mg/kg, i.m., twice daily) or saline for 12 days. Animals were killed at various times thereafter and their adrenal glands were analyzed for TH activity. Mean values are expressed as per cent of control (which averaged 36.5 ± 1.2 nmoles, ^3H_2O formed/hr/pair of adrenal glands). Numbers in parentheses indicate the number of animals in each group. There was no significant difference among control animals given saline injections twice daily for 4, 8 and 12 days. Therefore, their data have been pooled.

† $P < 0.05$, compared to control.

‡ $P < 0.01$, compared to control.

activity. Reduced sympathetic nerve activity may explain the hypotensive effect of long-term treatment with propranolol.

Male, spontaneously hypertensive rats, 6 to 7-weeks-old (Ralston Purina Chow Co., East Brunswick, NJ), were used. These rats were the progeny of the Kyoto–Wistar spontaneously hypertensive rats developed by K. Okamoto and K. Aoki in Japan. Rats were age and weight matched for each experiment; they were grouped six to a cage and were allotted treatment at random. All cages were kept under similar conditions of lighting and humidity in a room maintained at a temperature of 21 ± 0.5°C. Food and water were supplied *ad lib*.

(−)-Propranolol hydrochloride or its (+) isomer (2 mg/kg, i.m. twice a day) was administered to SHR daily for 8 or 12 days. Control SHR received equivalent volumes of saline (about 0.2 ml of 0.09% NaCl). Systolic blood pressure was measured in conscious rats 12 hr after the last injection of the drug by a tail cuff method, using the Narco Biosystem apparatus. Thereafter, animals were killed by a blow on the head and decapitated. Both adrenal glands were rapidly removed, cleaned, weighed and homogenized in 2.0 ml of ice-cold 0.25 M sucrose. The homogenate was centrifuged at 33,000 *g* for 20 min. A portion of the clear supernatent fluid was assayed for TH by the method of Levitt *et al.* [3] with modifications described in detail by Mueller *et al.* [4]. The enzyme activity was expressed as nmoles of ^3H_2O formed/hr/pair of adrenal glands. Tests of significance for the difference between the means of the different groups were performed by a two-tailed Student's *t*-test for unpaired data. $P < 0.05$ was regarded as significant.

Four days of (−)-propranolol treatment failed to affect adrenal TH activity or to alter systolic blood pressure (Table 1). When propranolol therapy was prolonged to 8 or 12 days, both adrenal TH activity and systolic blood pressure were significantly reduced. There was a correlation between the magnitude of decrease in enzyme activity and the degree of fall in systolic blood pressure. The antihypertensive effect of (−)-propranolol is related to β-adrenoreceptor blockade. Another pharmacological effect, that of membrane stabilization, does not appear to play a major role in the antihypertensive action, since 12 days of treatment with the (+) isomer of propranolol, which possesses minimal β-blocking activity but has the same membrane-stabilizing activity [5], was ineffective in reducing blood pressure or TH activity.

A fall in blood pressure does not always lead to a reduction in adrenal TH activity. Administration of drugs such as reserpine or phenoxybenzamine causes a lowering of blood pressure which is associated with an increase in adrenal TH activity [6–8]. It has been suggested that the effect of these drugs in increasing TH is a compensatory adaptation to the fall in blood pressure caused by them. The increase in TH activity by reserpine is due to an increase in sympathetic nerve activity, because interruption of sympathoadrenal activity at the ganglionic level by chlorisondamine, a long-acting ganglionic blocking agent, prevents reserpine induction of TH [9].

Thus, it appears that chronic treatment with propranolol causes a generalized decrease in sympathetic activity, including that of sympathoadrenal nerves leading to reduced TH activity. A decrease in activity of the rate-limiting enzyme in the biosynthesis of norepinephrine may lead to a decrease in the amount of amine available for release. Decreased sympathetic nerve activity coupled with a reduced amount of amine available for release would result in decreased vasomotor tone and hence a fall in blood pressure. This may explain the hypotensive effect of the drug in hypertensive patients.

The reduction in TH activity is a slow process; in the present study it took more than 4 days of treatment with (−)-propranolol before a significant decrease in enzyme activity was detected. This explains the delay in the onset of action of propranolol.

Note added in proof—While this manuscript was being prepared, a report [A. E. G. Reine and I. W. Chubb *Nature, Lond.* **267**, 265 (1977)] appeared which indicates that pretreatment of rabbits with propranolol for 6 days causes a significant reduction in tyrosine hydroxylase and dopamine β-hydroxylase activities of superior cervical ganglia. This is consistent with our results on the adrenal tyrosine hydroxylase activity of spontaneously hypertensive rats.

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Chemical nature of a methylmercury complex with a low molecular weight in the liver cytosol of rats exposed to methylmercury chloride

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Most of the mercury in the bile of rats exposed to methylmercury is found in two fractions, one bound to protein and the other bound to a small molecular compound [1–3]. The latter closely concerns the intestinal reabsorption of mercury excreted in bile [1, 3]. Several studies have been carried out to clarify the chemical nature of this mercury complex with a small molecular compound in the bile of the rat [1, 2, 4] and mouse [5]. A mercury fraction complexed with a small molecular compound, as well as a mercury fraction bound to protein, was also demonstrated in the cytosol of rat liver exposed to methylmercury [6]. A study of the chemical nature of the mercury complex with a low mol. wt within the liver cytosol might be valuable in understanding the transport mechanism of mercury from the liver cell to bile. We now present evidence that methylmercury glutathione is the predominant small molecular mercury compound within the liver cytosol.

Three female Wistar rats, weighing 190–200 g, were injected subcutaneously with methylmercury chloride (Wako Pure Chemicals, Osaka) at a dose of 2.5 mg, labeled with 125 μCi Me^{203} HgCl (930 mCi/m-mole, Radiochemical Centre, Amersham) per kg body wt. Methylmercury chloride was dissolved in 10 mM $NaHCO_3$–Na_2CO_3 buffer, pH 9.2. Two days after injection, the rats were killed after whole body perfusion under Nembutal anesthesia. The subsequent procedures were carried out at 0–4°C. Livers from three rats were pooled and homogenized with 3 vol of 0.32 M sucrose in 1 mM Tris–HCl buffer, pH 7.6. The homogenate was centrifuged for 10 min at 13,000 *g*, and the supernatant fraction was recentrifuged for 1.5 hr at

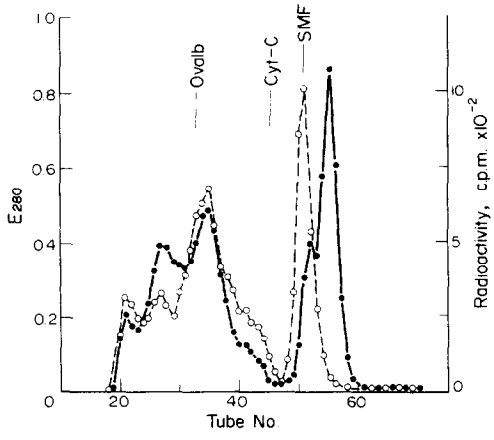

Fig. 1. Gel filtration on Ultrogel ACA-44 of liver cytosol prepared from rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μCi/Kg of Me^{203} HgCl for 2 days. Key: extinction at 280 nm (●——●); radioactivity, (○- - -○). Arrows indicate the position for ovalbumin (Ovalb), cytochrome *c* (Cyt-*c*) and the small molecular fraction (SMF).

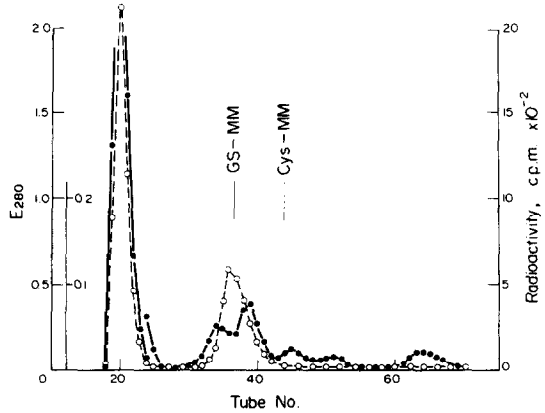

Fig. 2. Gel filtration on Sephadex G-25 of liver cytosol prepared from the rats treated with 2.5 mg/kg of methylmercury chloride plus 125 μgCi/Kg of Me^{203} HgCl for 2 days. Key: extinction at 280 nm (●——●); radioactivity (○- - -○). The left scale of E_{280} represents the extinction of tubes 18–24, and the right scale of E_{280} is for the extinction of tubes 24–69. Arrows indicate the position for methylmercury glutathione (GS-MM) and methylmercury cysteine (Cys-MM).