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SHORT COMMUNICATIONS

Glycerol-induced changes in human serum dopamine β-hydroxylase activity

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Dopamine β-hydroxylase (EC 1.14.17.1) catalyzes the conversion of dopamine to norepinephrine [1]. Serum dopamine β-hydroxylase (DBH) is considered to reflect the secretion of norepinephrine from the sympathetic portion of the peripheral autonomic nervous system [2–7], and it has been proposed that serum DBH may be an index of activity of the sympathetic nervous system [8]. Although this conclusion is not shared by several workers [9, 10], it is supported by the finding that serum DBH levels were elevated in patients with essential hypertension [11, 12], and decreased in patients with recent epileptic seizures [13]. It has also been reported that ethanol, a central nervous system (CNS) depressant, causes a decrease in both norepinephrine release [14–17] and DBH activity [18].

The serum levels of DBH vary greatly among normal human subjects, but each individual maintains the blood value of the enzyme at a relatively constant level [19, 20]. Age [20, 21], transient stress [22, 23] and genetic expression [24–26] have been implicated as factors which regulate serum levels of DBH in man. However, pharmacological agents are also known to influence the levels of serum DBH [9, 18, 27]; therefore, the identification of any agent that affects the circulating levels of this enzyme would contribute to our understanding of clinical problems which may be associated with changes in DBH activity.

In a recent report, Maclaren *et al.* [28] described a patient with an unusual CNS response to both orally and intravenously administered glycerol. As a result of that observation, a series of studies was initiated to determine the mechanism of this undefined intolerance to glycerol. In conjunction with these studies, the effect of glycerol on plasma DBH levels was investigated. The results of that investigation are presented in this report.

Glycerol (1 g/kg) (Fisher Glycerin diluted in 5 vol. of water) was given orally to six volunteer subjects and half the dose (0.5 g/kg) to a patient with an unusual CNS intolerance to glycerol. Blood samples were obtained just prior to the ingestion of glycerol and at 20-min intervals thereafter for 2 hr. Blood samples were also obtained from two other subjects, one after the intramuscular injection of insulin (0.075 units/kg) and another after the oral administration of leucine (0.15 g/kg). Serum was separated and kept at −70° until analyzed. DBH activity was determined using the method of Molinoff *et al.* [29] as modified by Karahasanoglu *et al.* [30]. To insure that alterations in DBH values were not related to an *in vitro* inhibition of enzyme activity by glycerol, rat brain and pooled human plasma DBH activity was measured in the presence of final concentrations of glycerol from 1.25 to 20.0 mM. Under these conditions there was no appreciable change in enzyme activity, and the optimum level of Cu^{2+} remained unchanged. In addition, samples obtained from a patient after glycerol ingestion were mixed with sera of known amounts of DBH in various proportions, and the expected enzyme activity was recovered. Samples for glycerol determination were collected in glycerol-free tubes, and glycerol was determined by a microfluorometric modification of a method using glycerol dehydrogenase [31].

The results summarized in Fig. 1 demonstrate that orally administered glycerol (0.5 to 1.0 g/kg) produced a significant increase in circulating levels of glycerol within 20 min, and by 40 min this concentration was more than 50 mg/100 ml compared to an initial value of 2.4 mg/100 ml. Concomitant with the rise in glycerol was a pronounced decrease in the serum DBH levels. The values are expressed as percentage of activity with respect to the initial value at 0 time. Although the initial values varied from 60 to 150 units of DBH, activity was found to be lower at all time periods in each of the six subjects after the administration of glycerol. In two subjects who did not receive glycerol (blood samples obtained during an insulin and a leucine tolerance test), DBH levels were essentially unchanged during similar time periods (Fig. 1). In a third subject, DBH levels rose during the course of a glucose tolerance test (results not shown).

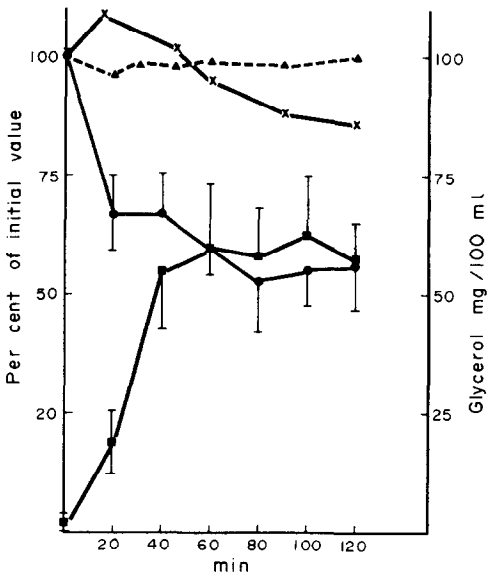

Fig. 1. Per cent decrease in serum dopamine β-hydroxylase activity after the oral administration of 0.5 to 1.0 g/kg of glycerol. Values are reported as the per cent change from the initial activity. Each point (●---●) represents the mean value ± S.E. for six human subjects (age range, 8–41). The initial DBH values were 60, 60, 71, 92, 113 and 150. Serum glycerol values (■---■) were measured on samples from five of these subjects, and the mean values ± S.E. for these results are also reported for each 20-min time period. The per cent change of serum DBH levels for two individuals not receiving glycerol are also reported: (1) (▲---▲) are values obtained during an insulin tolerance test, and (2) (×---×) are values obtained during a leucine tolerance test.

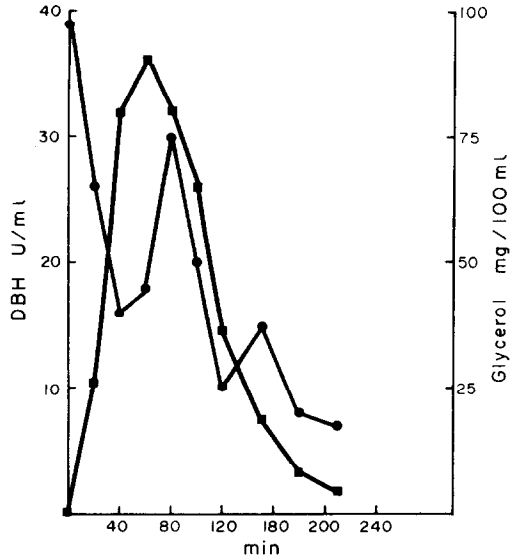

Fig. 2. Decrease in dopamine β-hydroxylase activity in a patient with intolerance to glycerol. Key: serum DBH (●---●) and glycerol (■---■) levels. The values represent the mean of duplicate determinations at each 20-min period for 2 hr and for each 30-min period for an additional 90 min.

The levels of DBH were also measured in a 4-year-old child who exhibited severe neurotoxicity to glycerol. In this instance, after the administration of glycerol, the levels of DBH decreased rapidly with a moderate rebound at 80 min followed by a continued decrease (Fig. 2). The DBH levels in this patient remained extremely low even after serum glycerol levels had decreased. The pattern of the oral glycerol tolerance test in this patient was different from that of the other individuals. In this patient with glycerol intolerance, the serum glycerol dropped more quickly, whereas in the six normal subjects, the glycerol levels were still elevated at 120 min. A similar observation was made with this child approximately 9 months earlier. At that time serum glycerol levels also rose to 50 mg/100 ml by 20 min. A decrease in DBH levels of more than 60 per cent was also observed during a 60-min intravenous glycerol tolerance test, but no significant changes in DBH levels were observed in this patient during the two separate 60-min glucagon tolerance tests.

The average range of variation for serum DBH activity in the same individual is relatively small [20, 32]. Although one report suggests that some fluctuations can occur over a 24-hr period [8], the results described above and by others [32] indicate that, in the absence of stress, serum DBH activity is relatively stable during a 2-hr period.

The results indicate that glycerol causes a pronounced decrease in the concentration of serum DBH. If DBH is a valid index of sympathetic activity, then the consistent 40–50 per cent decrease of activity in each of six individuals strongly suggests a relationship between this metabolite and activity of the sympathetic nervous system. This proposal is supported by the observation that glycerol produced severe central nervous symptoms in a young child [28] and that glycerol administered either orally or intravenously caused a rapid decrease in serum DBH levels in this child that was more pronounced than the decrease observed in the other individuals. At other times when this child was not receiving glycerol, the DBH levels were relatively constant.

Several laboratories have reported acute changes in circulating levels of DBH activity associated with a variety of stress conditions. One study reported a significant increase in serum DBH with exercise [22], although in other reports the increase was less [8, 23]. Changes in DBH after acute stress produced by the cold pressor test in man have also been reported [10]. In a recent report, Aunis *et al.* [32] suggested that an increase in DBH levels in 50 per cent of the individuals receiving theophylline might reflect an increase in peripheral catecholamine release. In most of these studies of acute stress, the change in DBH activity was small and variable. In the study presented above, glycerol ingestion caused a reduction of serum DBH levels in all of the individuals studied. In addition, this reduction was evident for each of the time periods when a sample was obtained, and the average decrease was approximately 50 per cent. Collectively, these data suggest an interrelationship between the activity of the nervous system and circulating glycerol.

It should be noted that several workers have questioned the usefulness of serum DBH as an index of sympathetic nervous activity [19, 33]. Indeed Reid and Kopin [9] have concluded that DBH activity does not necessarily parallel adrenergic activity or neurotransmitter release. They propose instead that plasma DBH is more closely correlated with the rate of synthesis of the enzyme in cell bodies and axonal transport, and it is possible that glycerol may affect these processes.

The exact mechanism whereby glycerol induces neurological symptoms is unknown [28]. Although recent studies have demonstrated some unusual kinetics for the oxidation of glycerol by brain slices and homogenates [34], there is no evidence for a direct connection between glycerol and an alteration in the activity of the nervous system. However, several reports indicate that ethanol can cause an inhibition of norepinephrine release in the brain [14, 17] and a decrease in DBH activity [18]. It is plausible that the effect of glycerol may be similar to that of ethanol. Studies are in progress to explore the possibility that glycerol exerts its neurotoxicity by interfering with the norepinephrine transmission of neuronal impulses.

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Predominance of the B form of monoamine oxidase in cultured vascular intimal endothelial cells

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Until recently the role attributed to endothelial cells has been that of a rather passive lining of blood vessels with little or no metabolic activity of physiological significance. This concept has changed gradually over the past 10 years due, in part, to the discovery that many biochemical and physiological events and systems are associated with endothelial cells from the intima of blood vessels. These findings include the demonstration of contractile elements [1], cell to cell communication [2], fibrinolytic activity [3], angiotensin conversion [4] and numerous drug and neurotransmitter receptors as well as cyclic nucleotide responses in intimal endothelial cells [5]. It has also been demonstrated that lung vasculature, including human lung, can alter the concentration of blood born vasoactive hormones as they transverse the pulmonary circulation [6–10]. Several recent reviews [11–15] have expounded upon the presumed role of the pulmonary vasculature in regulating the systemic circulating levels of a variety of vasoactive substances including angiotensin I and II, bradykinin, prostaglandins and biogenic monoamines. The principle site in lung responsible for these important processes is presumed to be the vascular intimal endothelium [11–15].

Pulmonary disposition of the biogenic vasoactive amines, norepinephrine and 5-hydroxytryptamine, has been extensively studied in a number of laboratories [6–15]. These reports have indicated that both norepinephrine and 5-hydroxytryptamine are actively transported into lung vasculature and subsequently degraded by the enzymes monoamine oxidase (MAO) and/or catechol-*O*-methyl transferase. The rapid appearance of metabolites of norepinephrine and 5-hydroxytryptamine in effluents from lungs perfused *in vitro* suggests that pulmonary amine uptake and degradation occur at or near the vascular luminal surface, presumably the endothelial cell [11–15]. Three functionally distinct forms of amine oxidase exist in the intact perfused lung [16]. Substrate and inhibitor studies indicate that two of the