Depression of Serotonin and Norepinephrine Levels in Brain Stem of Rabbit by Ethanol.* (25807)

DEHA GURSEY AND ROBERT E. OLSON

Dept. of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, Pa.

Physiologically active amines serotonin and norepinephrine are distributed in the brain in a regular pattern. High concentrations of both neurohormones are found in brain stem and low concentrations are found in cerebrum and cerebellum, and it has been suggested that serotonin is the chemical transmitter of the central parasympathetic system and that norepinephrine is the central sympathetic transmitter(1). The importance of these neurohumoral agents in those regions of the brain where autonomic regulation is believed to occur led us to study the effect of acute and chronic ethanol administration on their concentration in rabbit brain stem. A preliminary report has been made(2).

Materials and methods. White male New Zealand rabbits (4-6 kg) were injected intravenously with ethanol (2 g/kg in normal saline for 5-10 minutes) and then sacrificed at different intervals by intravenous injection of air. The brain was removed as rapidly as possible and either frozen at −5°C or homogenized with 0.1 N HCl. Serotonin was determined by the method of Bogdanski *et al.*(3) and norepinephrine by the method of Shore and Olin(4) using an Aminco-Bowman spectrophotofluorimeter.

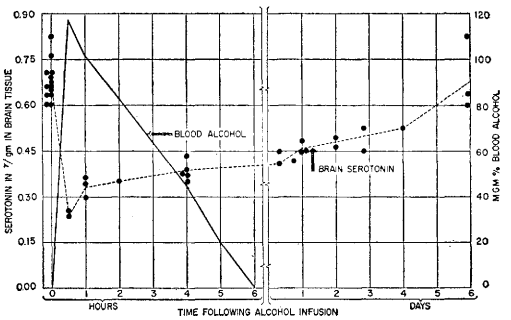

FIG. 1. Changes in blood alcohol and brain stem serotonin concentration in rabbits given 2 g/kg of alcohol for 10 min.

* This work was supported in part by grant from Nat. Inst. Health.

TABLE I. Parallel Changes in Serotonin and Norepinephrine Concentrations* in Rabbit Brain Stem after Ethanol.

Hr after ethanol	Serotonin γ/g	Serotonin % control	Norepinephrine γ/g	Norepinephrine % control
0	.67 (.60-.82)	100	.46 (.41-.51)	100
1	.33 (.30-.36)	49	.25 (.20-.34)	54
8	.42 (.40-.45)	62	.32 (.30-.34)	69
48	.46 (.45-.48)	69	.38†(.37-.40)	83
72	.49 (.45-.52)	73	.41†(.34-.48)	89
144	.69†(.60-.82)	102	.54†(.51-.56)	117

* Each value represents mean of determinations in 2-10 animals; mean and range are given.

† Avg not significantly different from control value at zero time. All other values different from value at zero time at less than 0.05 level of probability.

Results. Following acute injection of alcohol brain stem serotonin concentration declined rapidly to about 40% of control value at 30 minutes. The level then increased slowly reaching normal value in about 6 days (Fig. 1). Norepinephrine concentrations were reduced to about 55% of control values (0.46 μg/g) after 1 hour, then rose *pari passu* with serotonin concentrations to 100% of normal in 4-5 days (Table I). Shape and duration of both curves were very similar to patterns produced by intravenous injection of reserpine (5,6). Daily administration of ethanol (2 g/kg) intravenously to 4 rabbits for 7 days resulted in a significant depression of serotonin from a control average of 0.67 μg/g (range 0.60-0.82) to 0.32 μg/g (range 0.30-0.35) and a significant depression of norepinephrine from a control average of 0.46 μg/g (range 0.41-0.51) to 0.24 μg/g (range 0.20-0.26).

Discussion. This depression of brain stem levels of serotonin and norepinephrine by ethanol strongly resembles the depression produced by reserpine in that depletion persists long after the drug has disappeared. Blood alcohol levels were too low to measure 6 hours following acute dose, whereas depression of

brain stem serotonin lasted 15-20 times as long. This action of ethanol furthermore does not appear the result of hypnotic effect of the drug since common hypnotic and analgesic agents such as phenobarbital, barbital, morphine, and scopolamine do not lower brain stem serotonin levels in the rabbit(7). It has also been shown that duration of hypnosis in mice injected intraperitoneally with 4 g/kg of ethanol is potentiated by reserpine(8). Our observations that ethanol also produces an effect on the 2 neurohormones closely paralleling that of reserpine seem to provide the most reasonable interpretation for this potentiation.

A plausible hypothesis of neurohumoral action and the effect of reserpine upon these phenomena has been advanced by Brodie, Spector, and Shore(9). These authors postulate that binding sites for serotonin and norepinephrine exist in the brain stem, which provide a mechanism for controlled release of serotonin and norepinephrine in facilitating conduction. These authors furthermore attribute the sedative, hypotensive and other effects of reserpine to impairment of binding sites of serotonin and norepinephrine, which releases stored amines quickly and renders binding sites incapable of taking up newly synthesized amines. According to their hypothesis, these neurohormones are then liberated directly from a synthetic locus in the cell at a rate which may produce a physiologic effect if concentration at receptor site reaches a sufficiently high level. Under these conditions, rate of accumulation of amines will be dependent upon its rate of formation and its rate of inactivation by monoamine oxidase. For example, a slowly-formed amine may be metabolized as fast as it is formed while a more rapidly-formed amine may accumulate at receptor sites in concentrations sufficiently high to produce a response. It is known that brain serotonin is synthesized far more rapidly than brain norepinephrine and Brodie *et al.*(9) concluded that sedative effects of reserpine could be explained most readily by persistent bombardment of central receptor sites by free serotonin. The parallel depletion of both neurohormones and duration of the effect produced by ethanol are so similar to that produced by reserpine, that it is tempting to invoke the same mechanism to account for the hypnotic and sedative effects of the 2 drugs. This concept, if verified, would provide a physiological basis for the well-known mood depression of chronic alcoholics.

Summary. Evidence is presented which indicates that intravenous infusion of 2 g/kg of ethanol produced depression of both serotonin and norepinephrine levels in the brain stem of rabbit. The parallel depletion of both neurohormones persisted long after ethanol had disappeared, an effect which is reminiscent of the action of reserpine. Chronic administration of alcohol for 7 days produced 50% decrease in both serotonin and norepinephrine levels in rabbit brain stem.

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Received March 23, 1960. P.S.E.B.M., 1960, v104.