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Hypothalamic Function in Response to 2-Deoxy-D-Glucose in Long-Term Abstinent Alcoholics

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Background: The body adapts to diverse stressful stimuli with a response characterized by activation of the hypothalamic-pituitary-adrenal (HPA) axis. Chronic alcohol consumption can cause changes in the function of this neuroendocrine system. Although many studies have examined this phenomenon in drinking and recently sober alcoholics, few studies have examined HPA axis function in long-term sober alcoholics.

Methods: To characterize HPA axis function in long-term sober alcoholics, we used a challenge paradigm with 2-deoxy-D-glucose (2-DG). An infusion of 2-DG (a nonmetabolizable glucose analog) induces a well-characterized stress response. In a previous study, our laboratory found an exaggerated corticotropin and cortisol response in alcoholics abstinent 3 weeks; in this investigation we compared the effects of an infusion of 2-DG on 19 healthy volunteers and 20 community-living alcoholics who had been abstinent more than 6 months.

Results: In contrast to the previous study, long-term sober alcoholics did not have an exaggerated corticotropin and cortisol response after 2-DG.

Conclusions: Previously observed abnormalities in cortisol regulation in 3-week-sober alcoholics may be related to the acute effects of recent alcohol consumption and withdrawal. Future investigations into the metabolic function of alcoholics, particularly investigations involving the HPA system, should consider the possibility that normalization may not occur until long-term abstinence has been achieved.

Key Words: 2-Deoxy-D-Glucose, Cortisol, Corticotrophin, Alcoholism, Hypothalamus.

THE HYPOTHALAMIC REGION of the brain plays a central role in the response to physical and psychological demands that act to disrupt homeostasis. When such a condition, often described as stress, occurs, the hypothalamus initiates a neuroendocrine response that involves the pituitary gland and the adrenal cortex. The hypothalamus receives neural inputs from other brain regions, causing it to secrete corticotrophin releasing hormone (CRH), which stimulates the pituitary. The pituitary then releases corticotropin into the bloodstream, and this induces the adrenal cortex to secrete cortisol, a key hormonal mediator of the systemic response to stress. Cortisol has potent beneficial effects that help the body cope with physical injuries and adverse environmental conditions. However, when cortisol is increased for prolonged periods of time, adverse effects, such as Cushing's syndrome, can occur (Axelrod and Reisine, 1984).

Acute and chronic alcohol consumption can affect the hypothalamic-pituitary-adrenal (HPA) axis and the hormonal stress response. Some researchers have suggested that this interaction may facilitate some of the rewarding effects of alcohol consumption (Fahlke and Hansen, 1999) and have a role in premature aging associated with alcoholism (Spencer and Hutchison, 1999). In some situations, alcohol's effect on the HPA axis can be directly measured by analyzing plasma corticotrophin and cortisol. Plasma cortisol increases have been observed concurrently with alcohol consumption (Rivier et al., 1984; Wright, 1978) and also during the early alcohol withdrawal period (Adinoff et al., 1991). In abstinent alcoholics, baseline plasma cortisol generally returns to normal (Marchesi et al., 1994).

Persistent HPA functional abnormalities have been detected in abstinent alcoholics by means of experimental manipulations. A number of research paradigms, using a variety of stimulatory techniques, have been used to probe HPA axis function in these individuals. For example, an infusion of CRH has been used to stimulate the pituitary to release corticotrophin, and this in turn causes the adrenals to secrete cortisol (Holsboer, 1986). With this experimental paradigm, alcoholics have been found to have blunted cortisol or corticotropin release (Adinoff et al., 1990; Bailly et al., 1989; Inder et al., 1995; Loosen et al., 1991; von Bardeleben and Holsboer, 1988; von Bardeleben et al., 1989). However, a disadvantage of this technique is the fact that the hypothalamic portion of the HPA axis is bypassed.

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Research paradigms that examine HPA axis function by using dynamic testing overcome the limited scope of the CRH stimulation test. Dynamic testing of the HPA axis uses stressors that act at the hypothalamic level to activate all components of the axis (Ehrenreich et al., 1997; Knudsen et al., 1987). In alcoholics, dynamic studies have used a variety of stressful challenge paradigms, including insulin-induced hypoglycemia (Berman et al., 1990; Chalmers et al., 1978; Costa et al., 1996; Knudsen et al., 1987), operative trauma (Margraf et al., 1967), and physiologic stressors (Bernardy et al., 1996; Ehrenreich et al., 1997; Errico et al., 1993; Vescovi et al., 1997). These different paradigms have all shown that recently detoxified alcoholics have blunted cortisol or corticotrophin release in response to stress. A potential drawback of each of these paradigms is that they produce a definite, often profound, behavioral response that may itself affect the HPA axis.

When alcoholics are studied, this is potentially an important limitation because alcoholics or healthy volunteers could experience the behavioral effects in different ways on the basis of psychosocial distinctions or learned behavioral patterns. The alcoholic may be so accustomed to the sensation of stress that such experiences produce relatively less HPA activation when compared with a control subject. Thus, such dynamic paradigms might not be detecting alterations in isolated HPA function, but rather may be detecting group differences in the psychological perceptions of stress (Ehrenreich et al., 1997; Knudsen et al., 1987).

To overcome some of the limitations associated with prior studies, this laboratory has undertaken an investigation of HPA function in alcoholics by using a stressor that induces only a minimal behavioral response. This stressor is 2-deoxy-D-glucose (2-DG), a nonmetabolizable glucose analog that interferes with normal glucose catabolism and energy production (Brown, 1962). 2-DG inhibits glucose-6-phosphate dehydrogenase, blocking glucose breakdown and the production of adenosine triphosphate. As this process takes place, 2-DG gradually causes intracellular energy deprivation that results in HPA axis activation. 2-DG acts at the level of the hypothalamus to activate all the components of the HPA stress response. The effects of 2-DG can be quantified by measuring the subsequent increase in corticotrophin and cortisol (Weidenfeld et al., 1994). The unique benefit of this metabolic probe is that it causes only minimal subjective effects and therefore can be placebo controlled. Although an infusion of 2-DG can increase hunger, at low doses the overall physiologic response is subtle and not immediate (Breier, 1989; George et al., 1994).

In a previous study, this laboratory, as reported by George et al. (1994), gave an infusion of 2-DG to healthy volunteers and inpatient alcoholics who had been sober for 3 weeks. As expected, 2-DG induced a significant activation of the HPA axis that was associated with only minimal subjective symptoms. Considering the results of previous dynamic stress studies, a stress such as 2-DG could be expected to result in a blunted corticotropin and cortisol response in alcoholics. However, George et al. (1994) found that alcoholics had an exaggerated corticotrophin and cortisol response.

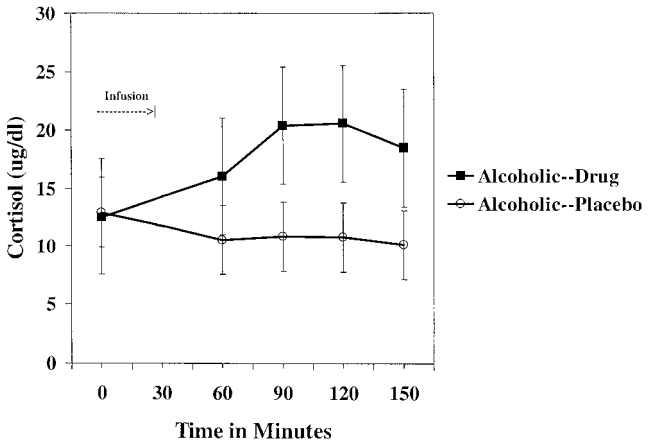

Fig. 1A. Effects of 2-DG administration and placebo on cortisol response in alcoholics.

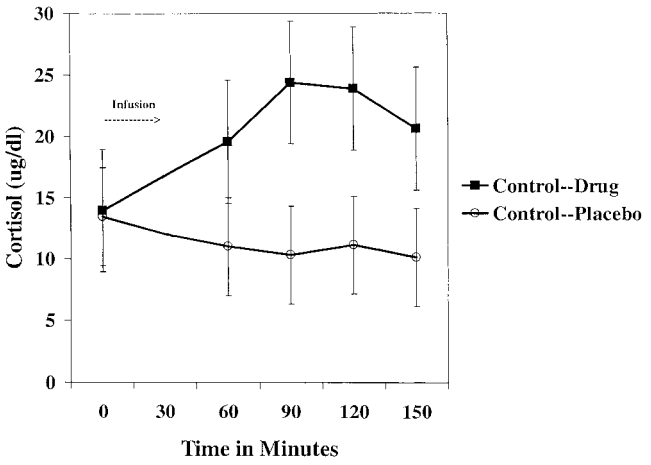

Fig. 1B. Effects of 2-DG administration and placebo on cortisol response in healthy volunteers.

It is possible that persistent acute effects of excessive alcohol consumption and withdrawal contributed to the heightened HPA activation noted by George et al. (1994). To evaluate this possibility, we elected to repeat the study in alcoholics who had been abstinent for more than 6 months. We reasoned that if the exaggerated cortisol and corticotropin response that George et al. (1994) demonstrated in 3-week-abstinent alcoholics was secondary to the prolonged effects of withdrawal, such a response would not be present in long-term sober alcoholics.

METHODS

Subjects

A total of 29 alcoholics, abstinent for more than 6 months, participated in the study. Alcoholics were recruited through local Alcoholics Anony-

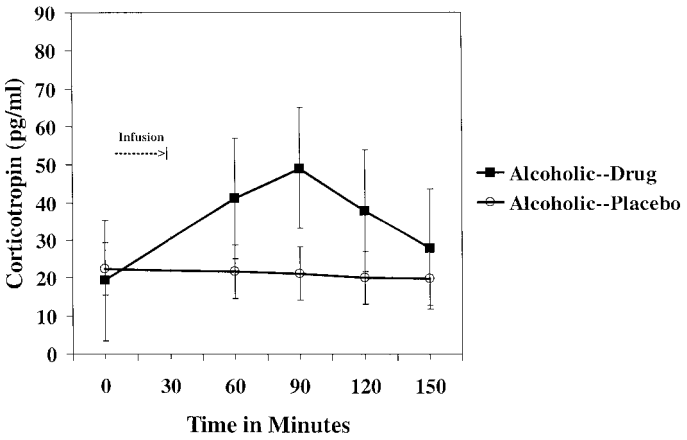

Fig. 2A. Effects of 2-DG administration and placebo on corticotropin response in alcoholics.

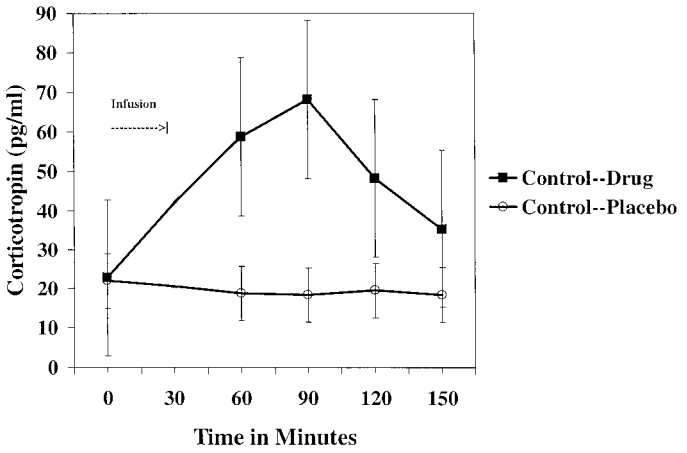

Fig. 2B. Effects of 2-DG administration and placebo on corticotropin response in healthy volunteers.

mous groups to match the demographical characteristics of the previous study. One alcoholic was recruited after his participation in the previous study (George et al., 1994). Verification of an individual's sobriety was obtained from Alcoholics Anonymous sponsors and from significant others. Nineteen male healthy volunteers were recruited from the Normal Volunteer Program of the National Institutes of Health in Bethesda, MD. All subjects underwent an extensive physical examination, including EKG, to ensure that they were in good physical health. All subjects had normal liver function tests. Subjects with a history of seizures or major head trauma (defined as a period of unconsciousness exceeding 1 hr) were excluded from participation. All subjects were medication free for at least 3 weeks before the study and had negative breath alcohol tests and urine drug screens. All healthy volunteers abstained from alcohol for at least 3 days before the study, and all alcoholics abstained for at least 6 months. Subjects with a history of intravenous drug abuse, schizophrenia, bipolar disorder, or organic brain dysfunction were excluded from the study. Subjects were reimbursed for their participation.

Psychiatric diagnoses were derived by using the SCID (Mazure and Gershon, 1979), which was administered by a social worker with extensive training in diagnostic interviewing. Drinking and smoking history were determined for each subject by means of a structured research questionnaire (Eckardt et al., 1978). Among the 19 healthy volunteers, 7 were nondrinkers. The other 12 were social drinkers who averaged one drinking occasion per week, averaging two alcoholic drinks at each occasion. Healthy volunteers did not fulfill DSM III-R criteria for depression or any Axis I diagnosis. Healthy volunteers also had a family history without significant alcohol abuse or dependence in parents or siblings.

Procedure

Written informed consent was obtained from each subject before inclusion in the protocol. The National Institute on Alcohol Abuse and Alcoholism intramural research program institutional review board approved the protocol. Participants were admitted to the Warren Grant Magnusen Clinical Center of the National Institutes of Health. After an overnight fast in the hospital, patients remained at bed rest and refrained from smoking. An intravenous flow was kept open with a slow infusion of saline for a stabilization period of 1 hr. Each subject then received either 25 mg/kg of 2-DG in 100 ml of normal saline or 100 ml of only normal saline (placebo) infused over 30 min. The two infusions were administered according to a randomly ordered, double-blind design. Each infusion was separated by at least 48 hr but by not more than 7 days. This paradigm approximated the conditions of our previous study (George et al., 1994). The subjects' temperature, blood pressure, and EKG were monitored throughout the study to safeguard against any adverse physiologic abnormalities. The infusions began at approximately 9:30 AM, after the circadian peak in ACTH and cortisol. Subjects were given a midday meal 150 min after the onset of the infusion.

Biochemical Variables

Blood samples were obtained through the intravenous line at baseline and at 60, 90, 120, and 150 min after the start of the infusion. These samples were immediately placed on wet ice, centrifuged, aliquoted, and stored at −70°C pending analysis. Covance Laboratory in Vienna, VA, performed the analysis. Corticotropin was assayed with immunoradiometric assay (IRMA) and had a 5.9% interassay variation, and cortisol was assayed with an enzyme-linked immunosorbent assay run on an Abbott (Abbott Park, IL) TDxFLx and had a 3.9% interassay variation.

Psychological Variables

Subjects completed a self-report rating scale at various time points during the study to quantify the perception of stress. A well-established measure, the Spielberger State Anxiety Inventory, was used to quantify anxiety (Spielberger et al., 1970). It was administered at baseline and at 150, 180, and 240 min after the start of the infusion. At 150 min, subjects completed the scales, indicating how they felt at the height of the infusion, and at 180 and 240 min, they indicated how they felt since the previous time they had completed the scales.

Statistical Analysis

The statistical package used for all analyses was Statistica by Statsoft (Tulsa, OK). Tests used included ANOVAs, MANOVAs, and correlations. A significance level of $p = 0.01$ was selected because of the number of tests performed. Results are expressed as mean ± SD.

RESULTS

All alcoholics fulfilled DSM III R criteria (American Psychiatric Association, 1987) for past alcohol dependence. Nine met DSM III-R criteria for past drug abuse or dependence. Alcoholics had a prior history of 10.9 ± 7.8 years of heavy alcohol consumption. They were abstinent for at least 6 months and had an average of 26.1 ± 25.3 months of sobriety before the study. The length of time that the alcoholics abstained from alcohol was not correlated with any biochemical or psychological variable. Similarly, the

history of drug use (including marijuana) was not related to any biochemical or psychological variable. Three alcoholics met DSM III-R criteria for depressive disorder, either at the time of the study or in the past; however, there were no group differences detected by the Hamilton Depression Index (Hamilton, 1967). Alcoholics were significantly different from healthy volunteers on all of the drinking variables we measured. Alcoholics also smoked significantly more cigarettes than did healthy volunteers. There were no group differences in age, weight, or liver function tests (Table 1).

Biochemical Variables

The 2-DG infusion caused a significant increase in plasma concentrations of cortisol and corticotropin. There was a significant interaction between drug and time for cortisol [$F(3,102) = 11.03$; $p < 0.0001$] and a drug × time interaction for corticotropin [$F(3,108) = 26.32$; $p < 0.0001$]. Interestingly, the cortisol and corticotropin response of the alcoholics was slightly decreased when compared with that of the healthy volunteers, but there were no significant differences between groups. When smoking status was added as a factor in the ANOVA, neither smoking nor any of its interactions with other factors were significant; this indicates that cigarette smoking had no effect (Figs. 1A, 1B, 2A, 2B).

Psychological Variables

There were no significant differences between the groups on the Spielberger State Anxiety Inventory. Anxiety in both groups was slightly higher after the 2-DG infusion, and it decreased after the meal, indicating a drug × time interaction [$F(2,70) = 7.30$; $p = 0.001$]. Mean scores ± SD at baseline for alcoholics were 30.60 ± 9.51 and for healthy volunteers were 28.39 ± 7.06. The scores were highest 150 min after the infusion, at which time mean scores ± SD for alcoholics were 32.74 ± 10.52 and for healthy volunteers were 32.68 ± 8.26.

Table 1. Age, Weight, Drinking Variables, and Liver Function in Healthy Volunteers and Alcoholics

Variable	Healthy volunteers ($n = 19$)	Alcoholics ($n = 20$)	ANOVA
Age (yr)	38.3 ± 9.0 (23–55)	40.5 ± 9.9 (23–56)	NS
Weight (kg)	82.4 ± 10.8 (62–102.1)	80.3 ± 17.2 (60.3–123.3)	NS
MAST	0.4 ± 0.8 (0–3)	38.6 ± 7.8 (23–51)	$p < 0.001$
Hamilton Depression Index	1.3 ± 2.5 (0–9)	2.6 ± 3.4 (0–12)	NS
Hollingshead	4.6 ± 0.6 (3–5)[a]	3.9 ± 0.9 (2–5)	$p < 0.01$
Average sobriety (months)	—	26.1 ± 25.3 (6–76)	—
Cigarettes per day	0.8 ± 3.4 (0–15)	7.5 ± 11.8 (0–40)	$p < 0.01$
Liver enzymes ALT (u/liter)	22.5 ± 8.3 (12–39)	20.8 ± 8.3 (7–47)	NS
AST (u/liter)	20.0 ± 4.2 (15–29)	21.5 ± 6.7 (13–35)	NS
Glutamyltranspeptidase (u/liter)	24.3 ± 9.7 (14–45)	29.1 ± 15.6 (14–69)	NS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NS, not significant.

Data are presented as mean ± SD (range).

[a] $n = 18$.

DISCUSSION

Chronic alcohol consumption is associated with changes in HPA axis function. Although many studies have examined this phenomenon in drinking and recently abstinent alcoholics, few studies have examined HPA axis function in long-term abstinent alcoholics. We are not aware of any studies that have examined all components of the HPA axis in long-term sober alcoholics. The results presented here demonstrate that the effect of a stressful stimulus on the HPA axis (including the hypothalamic component) is similar in long-term sober alcoholics and healthy volunteers. Although the Spielberger inventory detected higher levels of anxiety after the 2-DG versus placebo, these psychological differences were minimal. This suggests that the corticotrophin and cortisol release we measured in response to 2-DG was an innate reflection of HPA axis function and not a reflection of environmental and psychological factors.

Direct comparison between this study of long-term abstinent alcoholics and the previous study of recently abstinent alcoholics by George et al. (1994) is complicated by the fact that different individuals participated in the two studies. We attempted to minimize this potential problem by re-studying the alcoholics who had participated in the original study. However, most of the subjects from the first 2-DG study had resumed drinking, were employed full time, had moved, or were otherwise unavailable to participate in the study. Although it is possible that a sampling effect could explain the HPA axis functional differences between the two studies, we feel that this is unlikely because the alcoholics in both studies had similar demographical characteristics. The comparability of the two studies is also substantiated by neurochemical measures, which were similar in both studies. The alcoholic groups of both studies had similar baseline concentrations of corticotrophin and cortisol. The healthy volunteer groups of both studies had no difference in baseline concentrations of corticotrophin and no significant parametric or nonparametric differences at any time after the 2-DG infusion. Similarly, there were no significant baseline or overall differences in the cortisol response between the healthy volunteer groups of the two studies. Also, both studies had similar numbers of subjects, giving them both a power of 0.08 to detect an effect size of 1 SD.

Considering these facts, it is reasonable to suppose that

with extended abstinence, there is normalization of the exaggerated corticotropin and cortisol response to 2-DG that was noted by George et al. (1994). Other studies also suggest that alcohol-related HPA axis abnormalities resolve with extended abstinence. For example, Adinoff et al. (1990) used a CRH stimulation test to stimulate the HPA axis in alcoholics at various time points after withdrawal. He found an attenuated corticotrophin response in alcoholics abstinent 1 and 3 weeks, but he found that this same measure was normal in alcoholics abstinent 6 months or more. Ehrenreich et al. (1997) used a battery of physiologic stressors to dynamically stimulate the HPA axis and found that the attenuated increase in corticotrophin noted with 3 weeks of abstinence was normalized at 12 weeks. Compared with these studies, the distinctive feature of the 2-DG effect on the HPA axis is that in recently abstinent alcoholics, the response is exaggerated rather than attenuated. The reason for this difference is unknown but may be related to the unique nature of the stress caused by 2-DG.

In summary, our data suggest that with extended abstinence, the HPA axis seems to function the same in alcoholics as it does in healthy volunteers. The HPA functional abnormalities noted by George et al. (1994) in alcoholics abstinent 3 weeks are therefore most likely related to the effects of recent alcohol consumption and withdrawal. These findings parallel the findings of other research that shows that HPA axis function normalizes with extended abstinence (Adinoff et al., 1990; Ehrenreich et al., 1997; Marchesi et al., 1994). Future investigations into the metabolic function of alcoholics, particularly investigations involving the HPA system, should consider the possibility that normalization is still occurring up to 6 months after withdrawal from alcohol.

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