PERSISTENCE OF DEFECTIVE SEROTONERGIC AND GABAERGIC CONTROLS OF GROWTH HORMONE SECRETION IN LONG-TERM ABSTINENT ALCOHOLICS

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Abstract — In order to establish whether long-term abstinence from alcohol reverses the defective serotonergic and GABAergic controls of growth hormone (GH) secretion affecting alcoholic patients, the 5-HT1D serotonergic receptor agonist sumatriptan and the GABAergic agent gamma-hydroxybutyric acid (GHB) were administered to 12 normal men (32–49 years) and 22 non-depressed male alcoholic subjects (38–52 years) after 1–2 years of abstinence from alcohol. All subjects were also tested with placebos. Furthermore, tests with GH-releasing hormone (GHRH) and L-arginine (which releases GH from somatostatin inhibition) were performed to determine whether GH secretion in response to its major determinants is preserved in alcoholics. Administration of placebo did not change plasma GH levels in any subject. Similar GH responses were observed in normal controls and alcoholic subjects when GHRH or arginine were administered. A significant GH increase was observed in normal controls after sumatriptan or GHB injection; in contrast, GH secretion was not modified by sumatriptan or GHB administration in alcoholic patients. These data show a persistent selective loss of 5-HT1D receptor and GHB-mediated neurotransmissions in alcoholics that a long-term abstinence from alcohol is unable to restore.

INTRODUCTION

A variety of studies suggest alterations of serotonergic and gamma-aminobutyric acid (GABA)-ergic function in alcoholism (Sellers et al., 1992; Mhatre and Ticku, 1992); however, the precise mechanisms of these neuroendocrine disorders are still unclear.

There are various serotonergic receptor subtypes, which are involved in different ways in mediation of serotonergic neurotransmission (Bloom, 1990). The recent availability of the specific serotonergic 5-HT1D receptor agonist sumatriptan, which selectively stimulates growth hormone (GH) secretion in humans (Rolandi et al., 1992; Herdman et al., 1994), provided the possibility of gaining a better insight into this matter. In addition, the GABAergic agent gamma-hydroxybutyric acid (GHB), which has been found capable of stimulating growth hormone (GH) secretion in normal human subjects (Takahara et al., 1977; Gerra et al., 1994), could also be utilized in such studies.

We have recently identified defective serotonergic and GABAergic controls of GH secretion in alcoholics during the first 4 weeks of abstinence by using the sumatriptan test and the GHB test as biological indices of abnormal neurotransmission at the hypothalamic pituitary level (Coiro and Vescovi, 1995; Vescovi and Coiro, 1995).

Various neurological and neuroendocrine alterations have been observed in alcoholics studied during the first month of alcohol withdrawal. However, many changes appeared to be at least in part reversible after a long period of abstinence. In fact, alterations of hypothalamic–pituitary–adrenal axis function and various neuropsychological tests improve after long-term abstinence (Adinoff et al., 1990; Marchesi et al., 1994).

In the present study, in order to establish whether alterations in serotonergic and GABAergic control of GH secretion persist in alcoholics after a long period of abstinence, we tested the GH responses to sumatriptan and GHB in 12–24 months abstinent patients. In addition, the same subjects were tested with growth-hormone-releasing hormone (GHRH) and arginine [an inhibitor of

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somatostatin (Alba-Roth et al., 1988) to control the activity of the major regulators of GH secretion.

MATERIALS AND METHODS

Subjects

Twenty-two male chronic alcoholics aged 38–52 years with a previous history of continuous ethanol consumption of 10.2 ± 2.9 years (mean ± SEM), who had been abstinent from alcohol for 1–2 years as members of a multifamily group, were informed of the purpose of the study and gave their informed consent. The study was in accordance with the Helsinki II Declaration.

Clinical assessment

All patients were assessed with the Hamilton Rating Scale for Anxiety (HRSA) (Hamilton, 1959) and the Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960). HRSA and HRSD scores (mean ± SEM of 22 patients) were 8.0 ± 1.0 and 7.0 ± 0.5, respectively. All subjects had normal body weight [body mass index (mean ± SEM), 23.0 ± 0.8].

Two physicians performed clinical examination of the patients. Only subjects with normal or slightly altered liver function were included in the study. Particularly, the presence of hepatic abnormalities was assessed with laboratory tests (plasma levels of aspartate transaminase, 43 ± 15 [mean ± SD of 22 patients] UIK; alanine transaminase, 35 ± 14 UIK; gamma-glutamyl transpeptidase, 107 ± 32 U/l) and with liver, spleen, and portal vessel echography. Ultrasound examination and, in unclear cases, biopsy, excluded the presence of cirrhosis.

Twelve normal men (aged 32–49 years; body mass index 23.2 ± 1.3) participated in the study as controls. Control and alcoholic subjects showed normal plasma levels of free fatty acids (controls, 0.8 ± 0.075 mmol/l; alcoholics, 0.77 ± 0.08), measured with a colorimetric method using kits provided by Boehringer (Mannheim, Germany).

Each subject was tested five times within 4 weeks (sumatriptan, GHB, GHRH, arginine, and placebo tests). Tests were performed in random order at weekly intervals.

Experimental procedures

The experimental procedure was similar for all tests. At 09:00 on the day of the experiment, i.v. indwelling needles were inserted into antecubital veins of overnight fasting subjects in the recumbent position. The needles were kept patent with a slow infusion of normal saline (NaCl, 0.9% w/v); one needle was used for blood sampling and the other for GHRH, arginine, or saline administration. Sumatriptan was given s.c., whereas GHB was given p.o. In all tests, a basal blood sample was withdrawn 30 min after needle insertion, just before drug administration (time 0). Further blood sampling was performed at 15, 30, 45, 60, 75, 90, 105 and 120 min.

The tests were performed as follows. Sumatriptan test: 6 mg sumatriptan (Imigran; Glaxo, Verona, Italy) diluted in 0.5 ml distilled water was injected subcutaneously at time 0; GHRH test: 1 μg/kg body weight GHRH (1-29; Geref, Serono, Rome, Italy) diluted in 1 ml normal saline was injected as an i.v. bolus at time 0; arginine test: 30 g arginine monohydrochloride diluted in 50 ml normal saline was infused i.v. over a 30 min period from time 0; GHB test: 25 mg/kg body weight GHB (Alcover CT Sanremo, Italy) was given p.o. at time 0 after blood sampling; placebo test: an i.v. injection of 2 ml normal saline at time 0 was followed by the infusion of 50 ml normal saline over 30 min. In addition, 0.5 ml distilled water was injected s.c. at time 0 and a placebo was given p.o.

Blood pressure and heart rate were monitored at each sampling time in all tests.

Laboratory investigations

Blood samples were collected and centrifuged cold; plasma was stored at −20°C until assayed. Plasma GH levels were determined by radioimmunoassay (RIA) (Schalch and Parker, 1964) using commercial kits. All samples were analysed in the same assay and in duplicate. Sensitivity and intra-assay and inter-assay coefficients of variation were 0.5 ng/ml, 3.6% and 8%, respectively. Plasma insulin-like growth factor-I (IGF-I) and 24 h urinary cortisol levels were also measured, because both hormones are known to influence GH secretion (Dieguez et al., 1988). Plasma levels of IGF-I were measured in samples taken at time 0 in the sumatriptan, GHB, GHRH, arginine, and placebo tests. They were evaluated by RIA using kits obtained from Nichols Institute (San Juan Capistrano, CA, USA). Urinary cortisol level was
measured after extraction with dichloroethane in a sample collected over the 24 h preceding each test. Cortisol was determined by RIA with commercial kits (Amlec, Westbrook, ME, USA). In our laboratory, the normal range of urinary cortisol values is 80–250 nmol/24 h. Sensitivity of the assay was 2.8 nmol/l. Intra-assay and inter-assay coefficients of variation were 3.7% and 7.5%, respectively. The five values for plasma IGF-I and 24 h urinary cortisol obtained in each subject (before the five tests) were averaged. Each mean was considered the individual value for each subject and used for statistical analysis within each group.

Statistics

Statistical analysis was performed with Wilcoxon's matched-pair rank-sum test, the Kruskall-Wallis test, and ANOVA, as appropriate. Data are reported as the means ± SEM.

RESULTS

Basal GH levels were similar in control and alcoholic subjects (Fig. 1 and Fig. 2). In both groups, GHRH and arginine induced significant increments in plasma GH concentrations (Fig. 1). The GH responses to these two agents in the two groups were similar at all time-intervals examined. The administration of placebos (saline) did not change GH secretion in any subject (Fig. 1).

The administration of either sumatriptan or GHB to normal controls induced striking increments in plasma GH level ($P < 0.01$ at times 30, 45 and 60 min and $P < 0.05$ at times 75 and 90 min vs baseline) (Fig. 2). In contrast, sumatriptan or GHB administration did not significantly change plasma GH concentrations in alcoholic patients (NS vs baseline at any time point, $F = 28.20, P < 0.01$ vs normal controls for sumatriptan; $F = 28.80, P < 0.01$ for GHB) (Fig. 2). Plasma IGF-I levels were significantly higher in controls ($240 ± 20$ ng/ml) than in alcoholic subjects ($165 ± 13$ ng/ml; $P < 0.005$). Free urinary cortisol levels were similar in controls ($159.0 ± 18.0$ nmol/24 h) and alcoholic subjects ($155.2 ± 16.3$).

No blood pressure alterations or side-effects were observed after drug administration in any subject.

Fig. 1. Effect of saline, growth-hormone-releasing hormone (GHRH) or arginine administration on plasma growth hormone levels in abstinent alcoholics and controls.

Twenty-two alcoholics (ALC) abstinent for 1–2 years and 12 age-matched normal subjects (NC) were studied. Other details are in Materials and methods. Each point represents the mean ± SEM (bars).
Fig. 2. Effect of sumatriptan or gamma-hydroxybutyric acid (GHB) administration on plasma growth hormone levels in abstinent alcoholics and controls.

Twenty-two alcoholics (ALC) abstinent for 1–2 years and 12 age-matched normal subjects (NC) were studied. Other details are in Materials and methods. Each point represents the mean ± SEM (bars).

DISCUSSION

The results of the present study failed to show significant effects of either sumatriptan or GHB administration on GH secretion in alcoholics abstinent >1 year. These findings indicate that long-term abstinence from alcohol is unable to restore 5-HT1D-receptor- and GHB-mediated neurotransmission regulating GH secretion. On the other hand, the normal GH releasing effects of GHRH and arginine in alcoholics argue in favour of a preservation of the major determinants of GH secretion, i.e. GHRH and somatostatin. Therefore, alcoholism is not associated with alterations in the GH secretory machinery, but rather with disorders of the serotonergic and GABAergic controls of GH secretion. In agreement with these conclusions, Glue et al. (1988) showed blunted GH responses to clonidine in abstinent alcoholics, suggesting α-2-adrenergic receptor sub-sensitivity in alcoholism. Taken together, alterations in 5-HT1D-serotonergic and α-2-adrenergic-receptor-mediated control of GH secretion might represent different aspects of a common neuroendocrine disorder in alcoholics. In fact, studies by Conway et al. (1990) in the rat have shown that serotonergic neurotransmission mediates α-2-adrenergic-receptor-stimulated GH release.

The disorder affecting 5-HT1D-receptor-mediated neurotransmission in alcoholism might be part of a more general alteration of 5-HT1 receptors, whose function (particularly that of 5-HT1A and 5-HT1B subtypes) has been related to ethanol intake in the rat (for review, see Buczek et al., 1994). Nucleus accumbens, medial prefrontal and ventral hippocampus and dorsal raphe nuclei appear to be critical zones where these phenomena occur, because alcohol-related changes in 5-HT1A-receptor density have been described at these levels (for review, see Buczek et al., 1994). In the light of the present results, a chronic down-regulation in 5-HT1D receptors may be assumed to occur in the human alcoholic brain. The possible relationship of 5-HT1D-receptor changes with those of other serotonergic receptor subtypes, which are considered to play some important role in ethanol-seeking behaviour, requires further investigation.

Interestingly, as in alcoholics studied within a month of alcohol withdrawal (Coiro and Vescovi, 1995), our patients showed low IGF-I levels after many months of abstinence, suggesting a long-
term reduction of IGF-I anabolic activity after alcohol withdrawal. This phenomenon might delay the organic recovery during abstinence. The defective serotonergic regulation of GH secretion is likely to contribute to the significant decrease in circulating IGF-I levels observed in our alcoholics, because of the important role of serotonin in maintaining the 24 h episodic GH surges (Martin et al., 1978; Muller, 1987; McCann and Krulich, 1989) that control IGF-I production (Florini et al., 1985).

The finding of a persistent neurotransmitter disorder after so many months of abstinence suggests that the neurological damage produced by many years of alcohol consumption may be irreversible. On the other hand, both serotonergic (Le et al., 1981; Linnoila et al., 1987) and GABAergic (Mhatre and Ticku, 1992) neurotransmissions are intimately involved in the development of alcohol tolerance and dependence. Furthermore, serotonin is supposed to play a role in modulation of alcohol intake (Rockman et al., 1982; Daoust et al., 1984). Therefore, we cannot exclude that serotonergic and GABAergic alterations unmasked by sumatriptan and GHB persist after alcohol withdrawal because they are trait markers of alcoholism. If this was the case, the sumatriptan and GHB tests might contribute to the evaluation and follow up of alcoholics. Further studies are needed to verify this possibility.

REFERENCES


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