CLINICAL RESEARCH

Chlorpropamide alcohol flush and circulating met-enkephalin: a positive link

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Abstract

Chlorpropamide-alcohol flushing may be due to sensitivity to endogenous opiates. To investigate this possibility the plasma met-enkephalin and beta-endorphin responses to sherry with and without chlorpropamide were studied in six patients with non-insulin dependent diabetes and in six normal subjects. After chlorpropamide all patients showed a rise in met-enkephalin concentrations from a basal level of 50 ± 7.2 ng/l to a peak of 75 ± 8.1 ng/l ($p < 0.001$). In contrast, before chlorpropamide treatment was started met-enkephalin values did not change after alcohol. No significant changes in beta-endorphin values were observed. In six normal subjects pretreated with chlorpropamide the met-enkephalin concentration also rose from a basal level of 72 ± 15 ng/l to a peak of 103 ± 9.4 ng/l ($p < 0.002$). Again, the met-enkephalin rise was not observed after placebo. Neither beta-endorphin concentrations nor facial temperature changed significantly.

These data suggest that endogenous opiates may be implicated in CPAF. Furthermore, this is the first study in which a significant change in circulating met-enkephalin values has occurred.

Introduction

Some diabetics treated with chlorpropamide show facial flushing after alcohol. This chlorpropamide-induced alcohol flushing

(CPAF) is believed to be a dominantly inherited trait which has been reported to have a significantly higher prevalence in patients with type 2 (non-insulin dependent) diabetes than in those with type 1 (insulin-dependent) diabetes and healthy controls, who have a prevalence of CPAF of about 10%. Furthermore, the incidence of CPAF is much higher in type 2 diabetics with a strong family history of the disease.^{1,2} CPAF is associated with relative freedom from small and large-vessel disease.^{3,4} Others, however, have reported a much lower prevalence of CPAF and have been unable to confirm the association with diabetes.⁵⁻⁷ The wide differences in the reported incidence of CPAF may reflect the crudeness of the method of assessing the flush. The magnitude of the flush may also be different after long-term chlorpropamide treatment and after a single test dose.

The mechanism which produces the flush is poorly understood, and its blockade with aspirin⁸ and indomethacin⁹ might suggest a role for prostaglandins in its pathogenesis. Nevertheless, the fact that the flush can be blocked by the specific opiate antagonist naloxone and reproduced by an enkephalin analogue with opiate-like activity (D-Ala², Me Phe⁴, Met (O)-ol) enkephalin (DAMME)) has led to the hypothesis that increased sensitivity to endogenous opiates may play an important part in the pathogenesis of type 2 diabetes.¹⁰ It has also been suggested that the flush might be caused by enkephalin acting through a central mechanism subsequently leading to cutaneous vasodilatation or inhibition of tonic vasoconstriction, with a consequent rise in facial blood flow.¹¹

To investigate this further we studied the relation between chlorpropamide, alcohol, facial flushing, and the circulating levels of two endogenous opiate peptides, met-enkephalin and β -endorphin, in normal as well as diabetic subjects. These two opioid peptides have been shown chromatographically to circulate in normal people.^{12,13}

Subjects and methods

Six normal Caucasian subjects (3 men and 3 women) and six patients (4 men and 2 women) with type 2 diabetes were studied. The normal subjects received either 250 mg oral chlorpropamide or a placebo tablet, in a double-blind cross-over manner (with a period of

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four weeks between the two tests) at 2100 the day before the alcohol was given. In contrast, the diabetics were studied before and after four weeks of chlorpropamide therapy 250 mg daily; the dose on the day before the test was given at 2100 instead of the morning dose. On the day the test was performed an intravenous cannula was inserted four hours before the alcohol challenge. At 1200 the subjects attended the thermography department and were left to equilibrate for 30 minutes with the room temperature maintained at $20 \pm 1^\circ\text{C}$, humidity 40-50%. Two blood samples were taken at -10, and 0 minutes, after which the patients and subjects drank 40 ml of sherry over one minute, and further samples were taken at 10, 20, 30, 45, and 60 minutes. In the diabetics an additional sample was taken at 35 minutes. Samples were assayed for met-enkephalin (using a highly specific and sensitive radioimmunoassay¹⁴) and β -endorphin (using a C-terminal β -lipotrophin assay¹⁵).

The mean facial temperature was monitored continuously using the AGA 680 medical thermographic unit and recorded every minute from an area on the centre of the forehead (5×5 cm). The sensitivity of the system is 0.1°C at 30°C ; we recorded the mean temperature change on a digital analogue scale, 10 units equal to 0.1°C . Repeated experiments have shown that the forehead is the most stable area of the face at any one examination and also from time to time (S A Bowcock and E D Cooke, personal observations). A rise of 5 or more integration units was arbitrarily accepted as evidence of flushing. A thermographic image was recorded on 35-mm film and magnetic tape every five minutes.

All results are expressed as means $\pm$ SEM and were analysed with Student's *t* test for paired samples.

Results

In the six normal subjects pretreated with chlorpropamide there was a significant rise in the circulating plasma met-enkephalin concentrations at 20, 30, and 45 minutes with a mean basal level of 72 ± 15 ng/l rising to a mean peak at 30 minutes of 103 ± 9.4 ng/l ($p < 0.002$).

FIG 1—Met-enkephalin concentrations in response to 40 ml sherry in six normal subjects after 250 mg chlorpropamide (○—○) or placebo (●—●). Values are means $\pm$ SEM (normal range is 14-140 ng/l¹²).

In contrast, with placebo pretreatment there were no changes in plasma met-enkephalin concentrations after the alcohol challenge (fig 1). Concentrations of circulating β -endorphin and facial temperature did not change significantly in either experiment, and no subject experienced a feeling of flush.

After four weeks of treatment with chlorpropamide, the diabetics

showed significant rises in circulating plasma met-enkephalin concentrations in response to alcohol at 10, 20, 30, and 35 minutes (with a mean basal level of 50 ± 7.2 ng/l rising to a mean peak at 20 minutes of 75 ± 8.1 ng/l ($p < 0.001$); fig 2). No change in circulating plasma met-enkephalin concentration was observed when alcohol was taken alone (although five patients showed small decreases that were not significant).

FIG 2—Met-enkephalin concentrations in response to 40 ml sherry in six diabetics before (●—●) and after four weeks' chlorpropamide treatment (○—○). Values are means $\pm$ SEM (normal range is 14-140 ng/l¹²).

There was no significant difference in the basal met-enkephalin values between the diabetics and the normal subjects. In both groups the circulating plasma met-enkephalin responses to the alcohol challenge were significantly higher when chlorpropamide was given than in the control experiment.

The rise in plasma met-enkephalin concentration occurred in all the diabetics irrespective of flushing (five subjects were CPAF positive with a mean temperature rise of 17 ± 5.5 integration units (range 8-35) and one CPAF negative). The increase in plasma met-enkephalin concentrations did not correlate with the degree of flushing. There was no significant change in the plasma β -endorphin values.

Discussion

Chlorpropamide-induced alcohol flushing, in both diabetics and non-diabetics, can be blocked by the specific opiate antagonist naloxone and reproduced with an enkephalin analogue DAMME.¹⁰ We have shown here for the first time a significant rise in circulating plasma met-enkephalin concentrations in response to alcohol and chlorpropamide in normal subjects (who are CPAF negative) as well as non-insulin-dependent diabetics (both CPAF positive and negative).

The fact that met-enkephalin concentrations rose during the test in the CPAF-positive subjects suggests a possible role for met-enkephalin in the genesis of the flush in that these subjects show a degree of sensitivity of this opioid peptide. Lack of sensitivity to the action of met-enkephalin might, however, explain the failure of the CPAF-negative subjects (both diabetics and non-diabetics) to flush despite a significant rise in the circulating plasma met-enkephalin concentration.

The mechanism by which altered plasma met-enkephalin concentrations could result from a specific interaction between alcohol and chlorpropamide is unknown. CPAF may be associated with an abnormally large rise in plasma acetaldehyde

concentrations,^{16,17} which may be due to the inhibition of aldehyde dehydrogenase by chlorpropamide¹⁸; furthermore, it has been suggested that in vitro met-enkephalin can combine rapidly with acetaldehyde to form a stable adduct which changes its opiate biological activity considerably.¹⁹ If this were true in vivo possibly the acetaldehyde-enkephalin complex might change the half life of met-enkephalin, which might result in an increased plasma concentration after alcohol ingestion. The formation of such an adduct might change certain biological properties of met-enkephalin, leading to the production of a facial flush.

The origin of circulating met-enkephalin in man is not clear. It may derive partly from the adrenal medulla, where high concentrations are known to exist, or from the gut or neural plexuses, where it is believed to subserve a role as a neurotransmitter or neuromodulator. It is of interest, therefore, that this is the first experimental manoeuvre which has been shown significantly to change circulating immunoreactive met-enkephalin which occur in response to some interaction between chlorpropamide (or its metabolite) and alcohol appear, however, to be independent of the ability to flush or the presence of diabetes.

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Blood concentrations of acetaldehyde during chlorpropamide-alcohol flush

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Abstract

To test the suggestion that chlorpropamide-alcohol flushing (CPAF) resembles the disulfiram effect and might be mediated by acetaldehyde, the initial metabolite of alcohol, blood concentrations of acetaldehyde were measured after a drink of alcohol in controls and diabetics positive and negative for CPAF. The CPAF-positive diabetics had significantly greater blood acetaldehyde concentrations after alcohol than the CPAF-negative diabetics both with a single dose of chlorpropamide and after two weeks' chlorpropamide treatment. Concentrations in the CPAF-positive group after chlorpropamide were also significantly greater than after a placebo tablet. There was also a clear separation in the increase in facial temperature after two weeks of chlorpropamide between the CPAF-positive and CPAF-negative groups (although there was some overlap after a single tablet). There was no difference in plasma chlorpropamide or alcohol concentrations between CPAF-positive and CPAF-negative diabetics.

These findings show that CPAF is distinct from alcohol flushing and that the acetaldehyde concentration in the blood provides an objective measure of CPAF. The difference between flushing and non-flushing diabetics cannot be accounted for by differences in blood concentrations of chlorpropamide or alcohol.

Introduction

Many non-insulin-dependent diabetics treated with chlorpropamide show facial flushing after taking a small quantity of alcohol—chlorpropamide-alcohol flushing (CPAF).^{1,2} We have previously suggested that CPAF is inherited^{2,3} and that it may provide clues to the causes of non-insulin-dependent diabetes.⁴

In 1962 FitzGerald *et al* suggested that CPAF might be a disulfiram-like reaction mediated by acetaldehyde, the intermediate metabolite of alcohol.¹ Using the relatively insensitive methods of those days they found no significant difference in acetaldehyde concentrations in CPAF-positive and CPAF-negative diabetics. Recently a sensitive and precise method for