PAPERS AND ORIGINALS

Biochemical Evidence of Anxiety in Dental Patients

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Summary

Urinary metabolites before dental treatment were compared in a group of patients with dental phobia and in a matched control group. Plasma adrenaline, noradrenaline, and free fatty acids were estimated before treatment, immediately after sedation with diazapam 0·2 mg/kg body weight in the phobic patients, during induction of oral anaesthesia, and during and after surgery. Patients with dental phobia had significantly higher levels of adrenaline, which were only temporarily lowered by sedation, and which during treatment remained consistently higher than those of control patients.

Introduction

Many patients find operative dental surgery a stress-provoking situation, and in extreme cases this may amount to a "dental phobia" (Lautch, 1971). In an endeavour to determine the magnitude of this fear in biochemical terms plasma catecholamine levels were measured in patients undergoing dental surgery under local anaesthesia. The half-life of these hormones is measured in minutes; plasma levels therefore reflect the current level of anxiety and rapidly change with changing circumstances. A group of patients thought to be suffering from dental phobia were compared with patients known to tolerate routine dental treatment unsedated. To assess the duration of any differences metabolites of adrenaline and noradrenaline were measured in preoperative urine samples. Anxiety associated with anger and aggression is usually manifested biochemically by a rise in both adrenaline and noradrenaline production (Martin, 1961; Breggin, 1964). This is associated with mobilization of free fatty acids by noradrenaline (Taggart and Carruthers, 1971). Anxiety without these features, on the other hand, causes mainly a rise in adrenaline levels (Martin, 1961; Breggin, 1964). Plasma levels of free fatty acids and triglycerides were therefore also measured.

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Patients and Clinical Procedure

All patients volunteered to take part in the study, the purpose of which was explained. A phobic group of 11 patients with a specific fear of dentistry had been referred by their dental practitioners, who had found it impossible to carry out normal dental treatment. A control group of 11 patients was selected and matched for age, sex, and type of treatment. A further five volunteers who did not require sedation were sedated in the same way as the phobic group to determine the effect of diazepam in the presence of "normal" anxiety. Ages ranged from 16 to 50 years and all patients were medically fit. A full medical and dental history was taken from each patient. They were asked to save all urine passed for 24 hours before attendance for treatment.

As soon as the patient was seated in the dental chair an indwelling catheter was inserted into an arm vein under local anaesthesia. This was connected to an extension tube and three-way tap, and the whole assembly was covered with towelling so that the patient would not be aware of the taking of samples. A continuous E.C.G. recording was taken of lead I. After a minimum resting period of 10 minutes a pretreatment blood sample was taken. The 11 highly anxious patients were then sedated with diazepam 0·2 mg/kg body weight via the indwelling catheter, given at a rate of 10 mg/min. A further blood sample was taken from this group five minutes after the administration of the diazepam, at the time of maximum effect, and before making any attempt to approach the mouth. Further blood samples were taken from all patients during the administration of the local anaesthetic, which was 3% mepivacaine (Carbocaine) without adrenaline. Further samples were taken during the operative treatment and 10 minutes postoperatively. The dental procedures were standardized so far as possible. All patients were treated during an afternoon session by the same operator and in the supine position. The dentistry performed consisted of extractions and minor surgical procedures.

BIOCHEMICAL ANALYSIS

The metanephrine content of preoperative urine samples was measured by Pisano's (1960) method. Vanillyl mandelic acid was assayed semiquantitatively by paper chromatography (Robinson *et al.*, 1959). All blood samples were placed immediately in heparinized tubes containing sodium metabisulphite as an antioxidant and centrifuged at 5,000 r.p.m. for three minutes. A 5-ml sample of plasma was immediately pipetted into tubes containing 0·5 ml of iced 4N perchloric acid

and then frozen. Catecholamine analysis was carried out within 48 hours using a semiautomated fluorometric technique based on that described by McCullough (1968). It had previously been determined that blanks containing appropriate dilutions of diazepam did not fluoresce, confirming earlier observations (Carruthers *et al.*, 1970). Free fatty acids were measured by the sodium fluoresceinate method (Carruthers, 1972) and triglycerides by fluorometric analysis (Cramp and Robertson, 1968).

Results

The metanephrine levels and vanillyl mandelic acid content of preoperative urine samples were all within normal limits in both groups.

Plasma adrenaline levels showed significant differences between the two groups (Table I). The mean values in the unsedated control group showed no significant change other than a slight rise during the administration of the local anaesthetic and during the operative dentistry. The mean values in the phobic group were initially significantly higher ($0{\cdot}05 > P > 0{\cdot}02$) and remained so at all stages. They showed a significant fall after sedation ($P < 0{\cdot}01$) but even after this fall and before attempting treatment these patients still had higher adrenaline levels than were found in the control group at any subsequent time during dental treatment.

During administration of the local anaesthetic the adrenaline levels in the phobic patients rose to presedation levels, and during surgery they were always higher than those of the control group ($0{\cdot}10 > P > 0{\cdot}05$). At the conclusion of the procedure their adrenaline levels were still significantly higher than those of the controls ($0{\cdot}02 > P > 0{\cdot}01$). Variations in noradrenaline were not significant and are not reported.

The mean changes in heart rate in the two groups are shown in Table II. The mean values in the phobic group were higher at all stages. Immediately after the administration of diazepam there was a mean rise of five beats a minute. One patient in the phobic group developed ventricular extrasystoles during treatment. The five normal patients given diazepam showed no significant changes in adrenaline level on administration of diazepam but all showed a slight rise in pulse rate.

Discussion

Most of the patients who claimed to have a real fear of dentistry stated that they were aware of this for several hours before their appointment. Some stated that they had been unable to sleep the night before. In spite of this there was no evidence of increase in the production of catecholamines during this time as judged from urine analysis of their metabolites. Even samples taken immediately before treatment contained normal amounts of metanephrine and vanillyl mandelic acid. A rise in catecholamine production in response to this situation thus seems to be associated with the actual event rather than the anticipation of it.

The phobic patients in this study showed significant differences only in their adrenaline levels, which suggests that their response was one of pure anxiety without aggression. The fact that their free fatty acid and triglyceride levels remained within normal limits further supports this argument, since adrenaline has a far weaker effect in mobilizing free fatty acids than noradrenaline (Taggart and Carruthers, 1971).

Although intravenous diazepam makes dental treatment much easier it does not reduce the level of circulating adrenaline or the heart rate in phobic patients to normal, although there is a transient reduction. On the other hand, it was not possible to study phobic patients unsedated, and the plasma adrenaline might have risen to much higher levels under such circumstances. In favour of the view that diazepam has some beneficial effect is the fact that the most significant differences in mean adrenaline levels between the two groups were before the administration of diazepam and after treatment, when the effect of the drug was waning. Diazepam had little effect on the adrenaline level in the normal volunteers, which suggests that it lowers the level only when it is significantly raised by anxiety.

The rise in heart rate after administration of intravenous diazepam is consistent with the findings of Healy *et al.* (1970). Unlike that study, however, the rate did not subsequently fall below the predrug rate. This discrepancy may be accounted for by differences in methodology. Healy *et al.* measured the heart rate at specific intervals, whereas in this study the rate was measured at stages during the procedure which were thought to be particularly stressful. It is of interest to note that plasma adrenaline levels correlated well with the heart rate (Table III), particularly in the phobic group when the adrenaline levels were high.

TABLE III—*Correlation Coefficients (Plasma Adrenaline v. Heart Rate)*

Stage	Control Group	Phobic Group
Intravenous cannulation	0·2212	0·6123
Five minutes after diazepam sedation		0·6878
During administration of local anaesthestic	0·2226	0·8182
During dental surgery	0·0206	0·6741
10 minutes postoperatively and more than one hour after diazepam	0·1680	0·7664

A striking feature of this study was the contrast between the apparently tranquil state of the patients and the biochemical evidence of severe residual anxiety. The occurrence of at least one case of ventricular arrythmia suggests that while sedation with diazepam may facilitate treatment it is not entirely without

TABLE I—*Mean (± S.E.) Adrenaline Levels (μg/l.) in Two Groups of 11 Patients*

Sample	Control Group	Phobic Group	Significance of Differences Between Groups
Intravenous cannulation	Mean 0·45 (± 0·07)	Mean 0·72 (± 0·10)	$t = 2{\cdot}095$; $0{\cdot}05 > P > 0{\cdot}02$
Five minutes after diazepam sedation		Mean 0·58 (± 0·11)	
During administration of local anaesthetic	Mean 0·50 (± 0·10)	Mean 0·86 (± 0·15)	$t = 2{\cdot}008$; $0{\cdot}10 > P > 0{\cdot}05$
During dental surgery	Mean 0·51 (± 0·10)	Mean 0·79 (± 0·11)	$t = 1{\cdot}849$; $0{\cdot}10 > P > 0{\cdot}05$
10 Minutes postoperatively and more than one hour after diazepam	Mean 0·36 (± 0·09)	Mean 0·73 (± 0·12)	$t = 2{\cdot}487$; $0{\cdot}02 > P > 0{\cdot}01$

Normal values at rest 0·10 (± S.E. 0·10) μg/l. (M. Carruthers, personal communication, 1972).

TABLE II—*Mean (± S.E.) Heart Rates (Beats/Min) in Two Groups of 11 Patients*

Stage	Control Group	Phobic Group	Significance of Differences Between Groups
Intravenous cannulation	Mean 82 (± 0·11)	Mean 95 (± 0·20)	$t = 1{\cdot}888$; $0{\cdot}10 > P > 0{\cdot}05$
Five minutes after diazepam sedation		Mean 100 (± 0·17)	
During administration of local anaesthetic	Mean 88 (± 0·16)	Mean 98 (± 0·18)	$t = 1{\cdot}930$; $0{\cdot}10 > P > 0{\cdot}05$
During dental surgery	Mean 88 (± 0·15)	Mean 104 (± 0·19)	$t = 2{\cdot}200$; $0{\cdot}05 > P > 0{\cdot}02$
10 Minutes postoperatively and more than one hour after diazepam	Mean 75 (± 0·12)	Mean 88 (± 0·14)	$t = 2{\cdot}688$; $0{\cdot}02 > P > 0{\cdot}01$

the cardiovascular hazards that are associated with acute anxiety in this situation.

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References

Breggin, P. R. (1964). *Journal of Nervous and Mental Disease*, **139**, 558.
Carruthers, M. (1972). *Automation in Analytical Chemistry*, ed. L. T. Skeggs. In press.
Carruthers, M., Taggart, P., Conway, N., Bates, D., and Sommerville, W. (1970). *Lancet*, **2**, 62.
Cramp, D. G., and Robertson, G. (1968). *Analytical Biochemistry*, **25**, 246.
Healy, T. E. J., Robinson, J. S., and Vickers, M. D. (1970). *British Medical Journal*, **3**, 10.
Lautch, H. (1971). *British Journal of Psychiatry*, **119**, 151.
McCullough, H. J. (1968). *Journal of Clinical Pathology*, **21**, 759.
Martin, B. (1961). *Psychological Bulletin*, **58**, 234.
Pisano, J. J. (1960). *Clinica Chimica Acta*, **5**, 406.
Robinson, R., Ratcliffe, J., and Smith, P. (1959). *Journal of Clinical Pathology*, **12**, 541.
Taggart, P., and Carruthers, M. (1971). *Lancet*, **1**, 363.

Plasma Levels and Therapeutic Effect of 25-Hydroxycholecalciferol in Epileptic Patients taking Anticonvulsant Drugs

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Summary

Plasma levels of 25-hydroxycholecalciferol (25-HCC) were measured by a specific competitive protein-binding assay. Mean levels in both normal London adults and adolescent schoolchildren were 16 ng/ml and the mean level in a group of epileptic patients on high-dosage anticonvulsant therapy was 5 ng/ml, (difference from normals $P < 0.001$). Two further epileptic patients, with well-marked anticonvulsant osteomalacia, were treated with small doses of 25-HCC during full metabolic balance studies; rapid healing followed administration of 25-HCC by mouth in doses of 10-45 μg daily, which is well below the effective dose range of calciferol in this condition. These findings provided further evidence that anticonvulsant osteomalacia results from hepatic enzyme induction which, by increasing the metabolism of cholecalciferol to inactive compounds, lowers 25-HCC levels in patients whose dietary vitamin D intake and exposure to sunlight are otherwise adequate. Results also indicated that under certain circumstances 25-HCC may have considerably stronger antirachitic potency in man than has hitherto been recognized.

Introduction

The occurrence of rickets and osteomalacia among epileptic patients taking long-term anticonvulsant drugs in high dosage was first reported by Kruse (1968). A subsequent report confirming this observation postulated that hepatic enzyme induction was responsible for enhancing the hydroxylation of cholecalciferol (vitamin D_3) to inactive metabolites and thereby greatly increasing the patient's requirement for the vitamin (Dent, Richens, Rowe, and Stamp, 1970).

25-Hydroxycholecalciferol (25-HCC) is the major circulating metabolite of cholecalciferol. It is produced from cholecalciferol only by liver (DeLuca, 1969) and is the immediate precursor of 1,25-dihydroxycholecalciferol, which is now regarded as the biologically active, hormonal form of the vitamin (Kodicek, Lawson, and Wilson, 1970; Holick, Schnoes, and DeLuca, 1971; Lawson, Fraser, Kodicek, Morris, and Williams, 1971). It was therefore important to test whether circulating levels of 25-HCC were abnormally low in subjects receiving high doses of anticonvulsant drugs, as would be expected on the above premise, and also to test the possibility that administration of 25-HCC itself might therefore be appreciably superior to calciferol in the treatment of anticonvulsant osteomalacia.

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Subjects and Methods

Adult controls consisted of healthy academic and laboratory staff who were resident in London, and adolescent controls (in whom other plasma determinations were also performed) were boys, evenly divided between the ages of 11-17, in secondary schools under the Inner London Education Authority. The subjects or their parents were informed volunteers in the project. Epileptic subjects who were otherwise healthy were sampled from among the population of a Buckinghamshire epileptic colony whose diet contained adequate amounts of vitamin D (>70 IU daily) and who spent several hours a week out of doors. All were taking at least two of the major anticonvulsant drugs, each in high dosage. Two further epileptic patients whose case histories are recorded below had clinical evidence of osteomalacia, which was later confirmed on both biochemical and histological grounds.

25-HCC was measured by the assay of Haddad and Chyu (1971). Briefly, ether extracts of plasma were chromatographed on silicic acid columns to separate 25-HCC from cholecalciferol and from more polar metabolites. Aliquots of the 25-HCC fractions compete with ^{3}H(26,27)-25-HCC for binding to the 100,000-*g* supernatant prepared from rachitic rat kidney homogenates. Dextran-coated charcoal absorbs unbound tracer sterol, and the supernatant counts were compared with those from a standard curve prepared from ethanol solutions of crystalline 25-HCC (courtesy of Dr. John Babcock, Upjohn Pharmaceuticals Kalamazoo, Michigan).

Metabolic balance studies were performed according to standard principles (Reifenstein, Albright, and Wells, 1945) improved by the use of cuprous thiocyanate as an internal marker (Dick, 1969).