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Gabapentin augments whole blood serotonin in healthy young men

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Summary. It has been previously demonstrated that gabapentin, a gamma-amino butyric acid analogue, inhibits monoaminergic neurotransmitter release from rabbit caudate nucleus slices and from rat cortex. In humans this drug has been shown to have anti-epileptogenic activity. Serotonin may act as an inhibitory neurotransmitter and its interaction with blood platelets is thought to reflect its central actions. We investigated sleep stages, whole blood serotonin levels, and serum melatonin in healthy men after the administration of gabapentin. With increasing serum gabapentin levels six healthy subjects showed an increase in sleep stages 3 and 4 and in whole blood serotonin ($P < 0.05$). Serum melatonin levels were not influenced.

On account of these results we speculate that gabapentin modulates the release of serotonin from blood platelets. The increase in peripheral serotonin points paradigmatically to an increase in the bioavailability of serotonin which may account for the increase in sleep stages 3 and 4.

Keywords: Gabapentin, gaba-analogue, sleep pattern, human whole blood serotonin level, nocturnal serum melatonin profile.

Introduction

Recent investigations have shown that the administration of l-(aminomethoxy)-cyclohexane acetic acid (gabapentin), a structural analogue of gamma-aminobutyric acid (GABA), inhibits the release of catecholamines and serotonin but not acetylcholine after electrical stimulation of the nucleus caudatus (Reimann, 1983; Schlicker et al., 1985). Evaluation of the sleep pattern in healthy young subjects after the administration of gabapentin suggested an increase in sleep stages 3 and 4 (Clarenbach et al., 1986a, b). This effect is different from that of GABAergic drugs (i.e., benzodiazepines) which are known to decrease

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sleep stages 3 and 4. On the other hand increases in sleep stages 3 and 4 have been observed along with increased brain serotonin concentrations in the cat (Jouvet, 1969); however, it is difficult to extrapolate this finding to humans (Clarenbach et al., 1986a, b). As an approach to cerebral serotonergic mechanisms we investigated peripheral whole blood serotonin; the rational is the observation of the presence of specific binding sites in human platelet membranes. These binding sites appear to be involved in the transport of serotonin into cells; and serotonin accumulated in platelets accounts for more than 98% of whole blood serotonin. Not only serotonin but also its derivative melatonin may be involved in human sleep regulation (Cramer, 1974). On account of the similarity of central and peripheral (platelet) serotonin receptors, we speculate that gabapentin induces alterations in central serotonin metabolism (which may be reflected by changes in peripheral serotonin levels), and concomitantly, changes in sleep. We therefore investigated whole blood serotonin concentrations and serum melatonin levels in healthy men after the administration of gabapentin and examined these in relation to the sleep pattern.

Materials and methods

Six healthy men, aged 22–28 years, who did not take any other medication, received per os at 8:00, 14:00, and 20:00 h for three consecutive days either placebo, 200, or 300 mg gabapentin in a controlled, double blind, radomized crossover design. The nights 1 and 2 were considered nights of adaptation. Blood was taken and recordings were started at 22:00 h of day 3. The interval (wash-out phase) between each phase of medication lasted 4 days. Sleep stages were scored according to Rechtschaffen and Kales (1968).

Blood was drawn during the night of the 3rd, 10th, and 17th day from a venous catheder every hour from 23:00 to 6:00 into tubes containing EDTA or heparin. Two ml of blood was frozen in EDTA-coated tubes and kept at −82 °C for the determination of serotonin. Blood containing heparin was centrifuged for 10 min at 3,000 g. The plasma was aspirated and frozen at −20 °C for the determination of gabpentin and at −82 °C for melatonin.

Gabapentin was determined according to Hengy and Kölle (1985) after derivatization (Caudill et al., 1982). In serum gabapentin was stable at −18 °C for more than 6 months. The within-day precision was 2% in the concentration range measured. The lower limit of sensitivity was 10 ng/ml.

For the determination of serotonin, 0.2 ml thawed blood was mixed with 0.5 ml H_2O and kept for 10 min at room temperature. Protein was precipitated by the addition of 0.2 ml 10% (w/v) $ZnSO_4$ in 0.5 M NaOH (Geeraerts et al., 1974). The suspension was centrifuged for 2 min at 10,000 g. The supernatant was analyzed by high performance liquid chromatography using a nucleosil-C_{18}-column (Anderson et al., 1981). Serotonin was quantified amperometrically. The peak height was proportional to the concentration. The intra- and inter-assay coefficients of variation were 3.6 and 5.0%, respectively. The lower limit of sensitivity was 0.05 nmol/l.

Immunoreactive melatonin was determined with a radioimmunoassay (Brown et al., 1983; Rao and Mager, 1987). The intra-assay coefficient of variation was 12% (n = 10) using a concentration of melatonin of 0.3 nmol/l and 11% (n = 10) at 0.8 nmol/l. The inter-assay coefficient of variation was 10% (n = 7) over a period of 4 months. When increasing amounts of exogenous melatonin were added to the serum samples, the regression line passed through the origin after subtracting the endogenous immunoreactive melatonin. The lower limit of sensitivity of the assay was 0.1 nmol/l.

Assays were carried out in duplicate. Internal quality controls were run with each assay. We investigated whether a time effect was noticable as regards whole blood serotonin by an SPSS^x MANOVA programme which involved a 2-factorial analysis of variance with repetitive measurements. Since no time effects were noted, the effect of gabapentin medication was tested by the Wilcoxon rank test on the normalized data.

Results

Evaluation of the sleep EEG of the subjects disclosed that the total sleeping time, REM-sleep and REM-latency were not influenced by the administration of gabapentin; deep sleep latency of subjects treated with gabapentin was decreased to 70% compared to controls and sleep stages 3 and 4 were increased to 120% ($P<0.05$).

On the days when a total of 600 or 900 mg gabapentin was administered, the mean serum gabapentin levels were 25.1 and 16.4 µmol/l at 23:00 h; they dropped in the course of the night to 70 and 72%, respectively (Fig. 1) at 6:00 h. Serum gabapentin levels were stable between individuals.

A 2-factorial analysis of variance of the nocturnal whole blood serotonin profiles of the individuals on placebo or gabapentin revealed no effect of time. When individual's profiles were compared, serotonin levels (the average levels during the placebo, 600 mg, and 900 mg gabapentin treatment ranged from 0.53–1.15, 0.83–1.17, and 0.84–1.19 nmol/l, respectively), were elevated during treatment with gabapentin. This elevation could not be correlated with discrete phases of sleep, e.g., REM. Therefore whole blood serotonin levels in the same subject were averaged for further statistical analysis (Wilcoxon rank test).

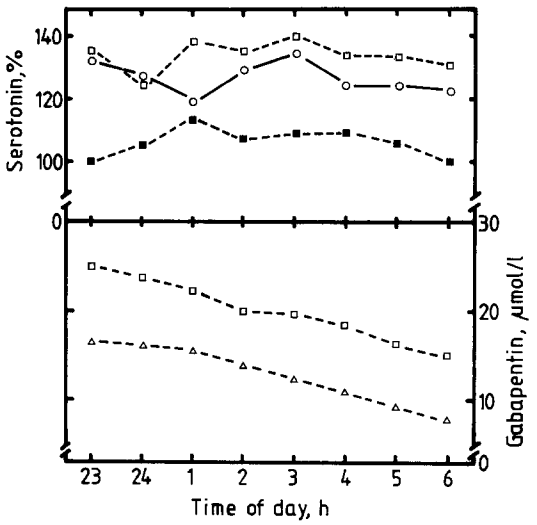

Fig. 1. Whole blood serotonin and serum gabapentin profiles in healthy men. The values are means from six subjects; the serotonin level is expressed in %, taking the concentrations at 23:00 h as 100. Whole blood serotonin: ■ – – – ■ placebo; ○———○ 600 mg gabapentin/d; □ – – – □ 900 mg gabapentin/d. Serum gabapentin: △ – – – △ 600 mg gabapentin/d; □ – – – – □ 900 mg gabapentin/d

Subjects on 600 mg gabapentin/d showed a 17% increase in serotonin level and on 900 mg gabapentin/d a 20% increase ($P < 0.05$).

At 2:00 h subjects on placebo or 900 mg gabapentin/d exhibited peak serum melatonin levels, they averaged 0.63 and 0.75 nmol/l, respectively, ($P < 0.05$). Subjects treated with 600 mg gabapentin/d had peak values (0.61 nmol/l) between 24:00 and 1:00 h. There were no consistent melatonin profiles in any of the subjects, i.e., peak values of one individual occured sometimes at 24:00, 1:00 or 4:00 h, or in another subject the zeniths of the three nights were congruent as regards time (Fig. not shown). Gabapentin did not influence the rhytmicity of the nightly melatonin surge. There was also no difference between serum melatonin levels during treatment with gabapentin or placebo ($P > 0.05$).

Discussion

It has been demonstrated previously that gabapentin prevented experimental convulsions in animals (Bartoszyk, 1983). On account of the observation that gabapentin inhibited the electrically evoked overflow of monoamines in a concentration-dependent manner (Schlicker et al., 1985) an interaction with GABA receptors might be feasible; however, the same authors observed that gabapentin's action was not influenced by bicuculline a drug acting directly on the $GABA_A$ receptor.

It is believed that the interaction of serotonin with blood platelets reflects its central actions (Sneddon, 1973; van Praag, 1977; Carlsson, 1976). If gabapentin inhibited the central release of serotonin, it might also inhibit the efflux of the transmitter from blood platelets, thus rendering the transmitter less susceptible to degradation and increasing its availability after stimulation. It is known that increased serotonin biovailability augments sleep stages 3 and 4 in experimental animals (Jouvet, 1969) and that REM sleep may be related to the nightly surge in melatonin (Sizonenko et al., 1979; Birkeland, 1982). Our observation in healthy subjects of increases in sleep stages 3 and 4 and no influence on REM sleep after the administration of gabapentin led us to investigate whole blood serotonin and serum melatonin levels. Gabapentin administration did not influence REM sleep in healthy subjects; this finding agrees with the observation that the serum levels and nightly profiles of melatonin were not altered. Treatment with gabapentin brings about an increase in whole blood serotonin; we believe that this increase reflects an increase in centrally active serotonin, which may be related to an increase in sleep stages 3 and 4. Further studies of the platelet model as a paradigm of central serotonergic sites involving the action of gabapentin are necessary to confirm these speculations.

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