Pharmacology Biochemistry & Behavior, Vol. 39, pp. 345–349. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

The Effect of Theophylline on Essential Tremor: The Possible Role of GABA

JUDIT MALLY[1] AND TREVOR W. STONE

Department of Neurology, Central Hospital of County Fejer, Szekesfehervar 8001, Hungary
Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ, Scotland

Received 20 September 1990

MALLY, J. AND T. W. STONE. *The effect of theophylline on essential tremor: The possible role of GABA*. PHARMACOL BIOCHEM BEHAV **39**(2) 345–349, 1991.—The chronic administration of theophylline was studied in twenty patients with essential tremor in a double-blind cross-over trial. The tremor was improved significantly after four weeks of treatment. In mice the chronic administration of theophylline was compared with propranolol on the modulation by adenosine, 5-HT, (−)isoprenaline or GABA of NMDA-induced depolarisation of neocortical slices. Adenosine depolarisation was abolished by two-weeks treatment with theophylline but not propranolol. Potentiation by (−)isoprenaline of NMDA responses was reduced by theophylline (100 mg/kg/day) and propranolol treatment (25 mg/kg/day), but a lower dose of propranolol further increased it. The enhancement by 5-HT of NMDA-induced depolarisation was unaffected by the pretreatment with theophylline, while the higher dose of propranolol blocked it. GABA caused no significant change of NMDA depolarisation in control slices, but after theophylline treatment (100 mg/kg/day) and propranolol administration at both doses it significantly potentiated NMDA depolarisation. The enhancement of GABA sensitivity might be an important common factor in decreasing the essential tremor after propranolol and theophylline treatment.

Theophylline Essential tremor GABA Adenosine Propranolol Neocortex Human Mouse

RECENT epidemiological studies have concluded that essential tremor is the most frequently occurring movement disorder (1,18). The neurological disease is characterised by a tremor mainly under postural and active conditions which is slowly progressive over the years.

The patients with essential tremor can be divided into subgroups which respond moderately to different drugs. A proportion of patients with alternating tremor burst in antagonists may not respond to propranolol (5). Many of the patients with essential tremor can be treated successfully with either propranolol or primidone but in a few cases the effectiveness of the therapy is restricted by the side effects of these drugs (7,13), particularly the negative inotropic effect and sedative effects (7, 10, 27).

Despite its ability to induce tremor in appropriate dosage (3,24), we have recently reported that theophylline in low doses might be useful in the treatment of essential tremor (16,17). In this study we have investigated the effect of low dose theophylline on essential tremor and compared it with that of placebo, in a double-blind cross-over study. The therapeutic range of theophylline in the serum has been defined but, since central action of theophylline has been implicated in essential tremor, the concentration in cerebrospinal fluid has also been measured.

In animal studies we have also attempted to investigate which kind of neurotransmitter systems are involved in the effect of chronic theophylline administration in the central nervous system (CNS). Although theophylline is widely recognised as an antagonist at adenosine receptors in the peripheral and central nervous systems (21), there are reports from binding studies of changes in other receptors in the CNS following chronic caffeine or theophylline treatment (8,25). We wanted to know which kind of neurotransmitter changes in CNS might be important in the reduction of essential tremor after theophylline administration. We compared this treatment with chronic propranolol treatment, which is widely used in essential tremor, to find a similar change in receptor sensitivity, which may explain, in part, the reduction of essential tremor. The long-term relief of essential tremor by propranolol is thought to be connected with the effect of the drug on the CNS, but the site of action within the CNS is poorly defined.

In this respect we have investigated the neuromodulatory effect of adenosine, 5-hydroxytryptamine (5-HT), (−)isoprenaline and gamma-aminobutyric acid (GABA) before and after the chronic administration of theophylline and propranolol upon the N-methyl-D-aspartate (NMDA)-induced depolarisation of the mouse neocortical slice preparation.

METHOD

Clinical Studies

Twenty outpatients with essential tremor were involved in this study. Their ages ranged between 19 and 88 (average: 68). Duration of their disease was between ½ and 10 years. Six of the patients had a family history of essential tremor. Criteria of the investigation was that no other disease existed and no other medicines were given. The patients were nonsmokers. No sign

[1]Requests for reprints should be addressed to Judit Mally, Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ, Scotland.

of abnormal liver and kidney function was found in clinical laboratory tests. All patients were fully informed about the purpose of this study and gave their voluntary consent.

Sustained release theophylline or placebo tablets were administered in a double-blind cross-over manner. Theophylline tablets (Theophtard, BIOGAL Pharmaceutical Factory, Debrecen) and placebo tablets were given once a day orally in the morning. All subjects underwent a control period without drugs before the trial when tremor was measured on five different occasions. During the treatment periods the tremor was assessed twice a week for at least 8 weeks. Serum and samples of CSF were taken before the ingestion of the tablet. In ten cases cerebrospinal fluid was taken by cisternal puncture during the steady state of the treatment.

Tremor Analysis

Tremor was evaluated by a volumetric method which is well correlated with the clinical score, but allows the quantitative assessment of tremor.

The patients were asked to hold a 100 ml plastic cup filled to the brim with water (top diameter: 3.2 cm; bottom diameter: 4 cm; height: 6 cm; weight: 1.93 g). The patient held the cup in semiflexed position of the hand for 60 seconds. The water left in the cup was measured after one minute. This was repeated ten times at each assessment and the mean values calculated.

The serum theophylline concentration was measured by radioimmunoassay using a kit obtained from the Institute of Isotopes of the Hungarian Academy of Sciences.

Experimental Studies

Male mice were injected with saline as a control group and either theophylline (10 mg/kg/day or 100 mg/kg/day) or propranolol (5 mg/kg/day or 25 mg/kg/day) for two weeks.

The animals were killed by cervical dislocation and the brain removed into cold, oxygenated bicarbonate buffer medium of the following composition (mM): NaCl 115; KCl 2; KH_2PO_4 2.2; $CaCl_2$ 2.5; $MgSO_4$ 1.2; NaH_2CO_4 25; glucose 10. Using a vibratome, sections of brain were cut 500 μM thick in a coronal plane to include regions of fronto-parietal and cingulate cortex as described in detail previously (2). These slices were then trimmed and transferred to two-compartment chambers where they were positioned across a greased slot in the dividing wall so that most of the grey matter lay on one side, while the white matter extended into the second chamber. Silver/silver chloride electrodes in contact with the solutions on the two sides of the dividing wall were used to record DC potentials which were displayed on a digital oscilloscope and chart recorders.

Both compartments were superfused with the bicarbonate buffer. NMDA was added into the superfusing fluid for one-minute periods. When applied alone to neocortical slices neither adenosine, 5-HT, GABA nor (−)isoprenaline had any significant effect alone. A depolarising agent, NMDA, at a concentration of 20 μM was chosen to examine the possible modulation of sensitivity of these neurotransmitters on induced depolarisation. The test drugs were perfused for ten minutes.

Statistical analysis of the clinical results was performed with a Wilcoxon Matched-Pairs signed-rank test at the end of 4-weeks treatment with theophylline or placebo and at the end of a further 4-weeks cross-over treatment. The results of the experimental work were analysed by an unpaired *t*-test.

RESULTS

Clinical Studies

After a single oral dose of 300 mg theophylline the tremor in ten patients was followed for 24 hours. No significant change was observed in the quantity of tremor assessed by the volumetric method. The concentration of theophylline in the serum increased slowly and reached its maximum level between 5 and 7 hours.

Twenty patients with essential tremor were treated with 300 mg theophylline or placebo tablets. After seven days treatment in ten patients starting with theophylline (300 mg/day) the tremor decreased gradually up to the end of the fourth week ($p<0.05$) (Fig. 1). From the fifth week the tablet was changed to placebo. Assessed by the volumetric method, the tremor deteriorated after two weeks administration of placebo. The worsening was significant but still these values were higher than those in the control period (Fig. 1).

In the reverse paradigm ten patients starting with placebo showed no change in the degree of tremor, but as the theophylline therapy was started a significant improvement of tremor was observed after four weeks (Fig. 2).

The steady state concentration of theophylline in serum was reached after one week; this was always under 10 mg/l with an average 3–5 mg/l. In the fourth week, in ten cases the concentration of theophylline was measured in both serum and CSF. The correlation coefficient was r = .92. The ratio of serum:CSF was 0.30 (Fig. 3).

Experimental Studies

The modulation of depolarisation by NMDA was studied with adenosine (20 μM), 5-HT (10 μM), (−)isoprenaline (200 nM) and GABA (100 μM) before and after the chronic administration of theophylline (10 and 100 mg/kg/day) and propranolol (5 and 25 mg/kg/day).

Adenosine potentiated the NMDA-induced depolarisation by 48.81% ± 10.0 (n = 21; $p<0.001$) compared to control. After the administration of theophylline for two weeks at either of the two doses the potentiation was abolished. Following the chronic administration of propranolol with either 5 mg/kg/day or 25 mg/kg/day the adenosine response was not changed.

(−)Isoprenaline (200 nM) enhanced the NMDA depolarisation by 40.2 ± 8.4 (6). This was prevented by theophylline pretreatment at 100 mg/kg/day, but not at 10 mg/kg/day (Table 1). Administration of the highest dosage of propranolol 25 mg/kg/day for two weeks caused a similar reduction in the induced depolarisation as the pretreatment with theophylline (Table 1). The lower dosage of propranolol further increased the potentiation of depolarisation compared to (−)isoprenaline alone (Table 1).

5-Hydroxytryptamine (10 μM) also enhanced significantly the depolarisation produced by 20 μM NMDA (Table 1). Propranolol treatment for two weeks in the higher dosage suppressed this effect of 5-HT. Theophylline administration had no influence on increased depolarisation by 5-HT either at 10 mg/kg/day or 100 mg/kg/day dosages (Table 1).

GABA (100 μM) did not change the induced depolarisation of NMDA compared to control. However, after chronic administration of propranolol in both dosages, the depolarisation produced by NMDA was enhanced by GABA (Fig. 4) (22.0% ± 7.82, n = 6, $p<0.01$; 40.07% ± 15.44, n = 5, $p<0.05$).

A similar effect was observed after theophylline 100 mg/kg/day treatment (23.15% ± 8.40, n = 6, $p<0.05$). GABA sensitivity did not change after theophylline 10 mg/kg/day administration for two weeks (Fig. 4) (9.4% ± 11.25, n = 5, n.s.).

DISCUSSION

The results of the double-blind cross-over trial showed that the low dosage of theophylline is also effective in reducing the

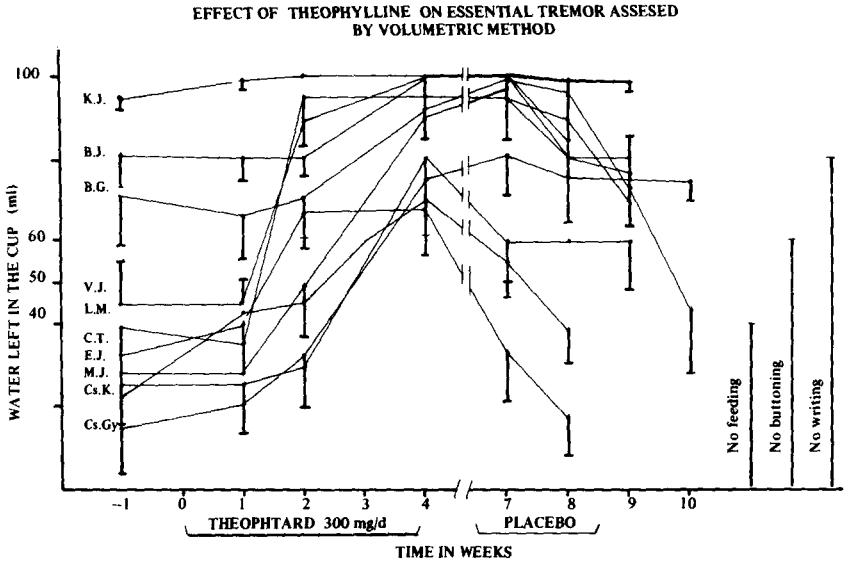

FIG. 1. Changes in the quantity of tremor assessed by a volumetric method in ten patients starting with theophylline 300 mg/day. Tremor in the most severely affected hand is shown. The reduction of tremor at the end of the fourth week was significant ($p<0.05$).

essential tremor. The therapeutic serum level of theophylline in essential tremor is far less than is recommended in asthma bronchiale. We observed measurable concentrations of theophylline in cerebrospinal fluid during the steady state with a CSF:serum ratio of 0.3.

Patients with predominant postural essential tremor, independent of the duration of the disease or its severity, reacted well after two weeks of theophylline 300 mg/day therapy. No acute effect was observed of theophylline 300 mg per os even though the concentration of theophylline in serum was about 5 mg/l during the steady state. The beneficial effect of theophylline developed slower than that of propranolol on essential tremor (4, 6, 11). Adverse effects of theophylline have been reported previously in hyperthyroidism and asthma bronchiale, but the concentration of theophylline in serum was above 10 mg/l in those cases (3,24).

Interpretation of the controversial alteration of tremor by theophylline is difficult if we take account of only one receptor site. The changes in the number of different receptors after chronic administration of caffeine or theophylline have been

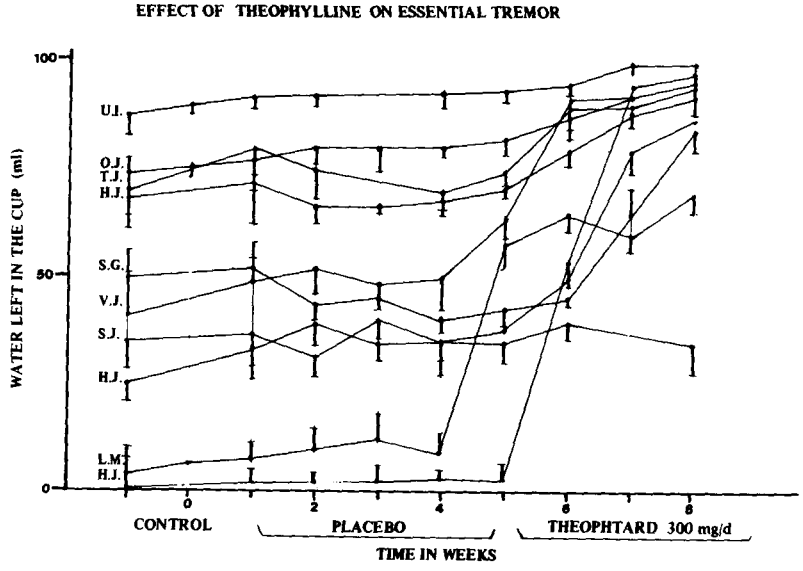

FIG. 2. Changes in the quantity of tremor assessed by a volumetric method in ten patients starting with placebo. No significant reduction was observed during this period.

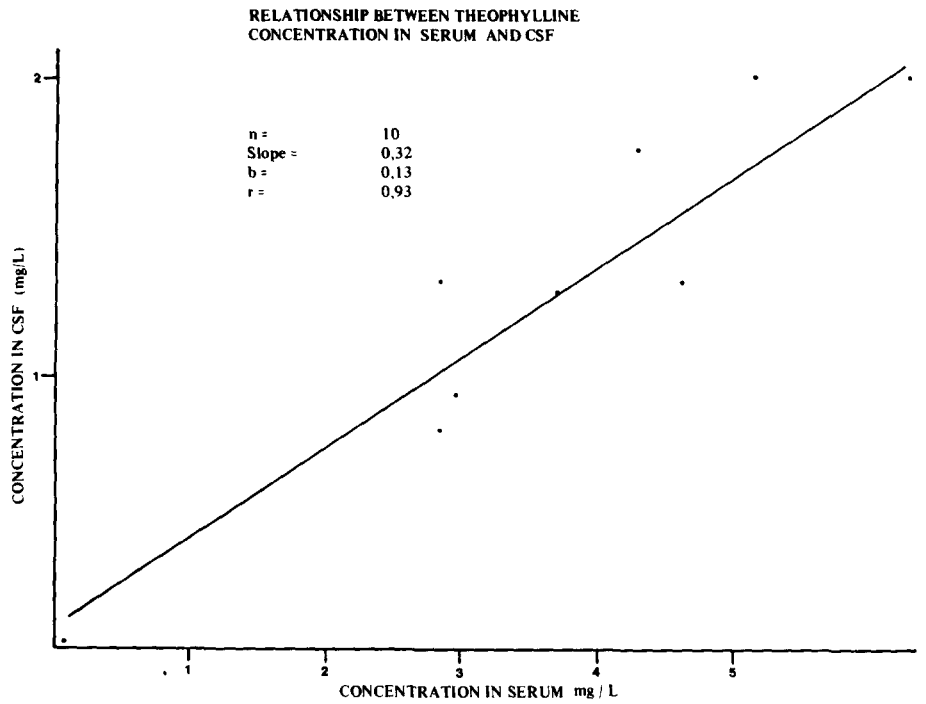

FIG. 3. In ten cases the CFS and serum theophylline concentrations were compared. A close correlation was observed (r = .93).

shown by binding studies. After two-weeks pretreatment with xanthine derivatives an upregulation of A1 receptors (21) and a similar upregulation of benzodiazepine binding sites (25) has been reported, while a decreased number of beta-adrenoceptors in hippocampus has been observed (8). The increased number of GABA receptor sites would be entirely consistent with the functional upregulation of GABA presented in this study.

This work therefore raises the possibility of a role for GABA in decreasing the tremor following the chronic treatment with propranolol. The acute effect of propranolol in essential tremor is considered to involve block of the physiological tremor by antagonism at peripheral nonselective beta-adrenergic receptors (26). The role of beta-adrenoceptors in the central nervous system in the explanation of the relief of essential tremor by propranolol is controversial since not all beta-adrenergic inhibitors are effective in essential tremor (9, 12, 14, 22, 27). This is certainly attributable partly to poor penetration across the blood-brain barrier by some of these drugs, but pharmacological factors may also be involved.

After two-weeks treatment with propranolol at either 5 mg/kg/day or 25 mg/kg/day doses, an upregulation of brain GABA

TABLE 1

ENHANCEMENT OF DEPOLARISATION RESPONSES TO NMDA INDUCED BY (−)ISOPRENALINE OR 5-HT IN RAT CORTICAL SLICES

	Enhancement of NMDA Responses (%)	
Pretreatment	(−)Isoprenaline	5-HT
Control	40.2 ± 8.4 (6)*	38.25 ± 6.12 (6)
Propranolol 5 mg/kg/day	64.6 ± 9.35 (7)†	27.03 ± 8.33 (6)
Propranolol 25 mg/kg/day	10.42 ± 5.56 (6)†	10.21 ± 9.13 (6)†
Theophylline 10 mg/kg/day	38.20 ± 19.9 (5)	57.5 ± 6.5 (6)
Theophylline 100 mg/kg/day	12.5 ± 4.5 (10)†	39.0 ± 13.2 (7)

*Data are shown as mean ± s.e.m. (for n slices).
†Significantly different from corresponding control, $p<0.05$.

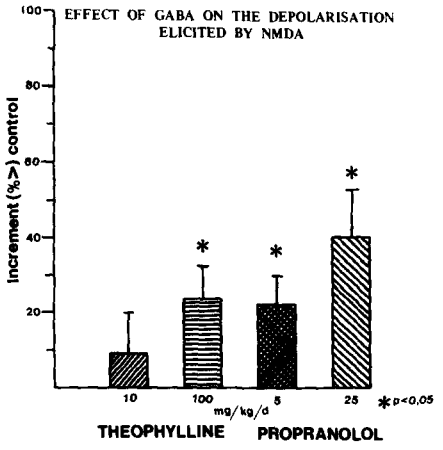

FIG. 4. Histogram summarising the GABA-induced enhancement of NMDA-induced depolarisation of neocortical slices after theophylline (10 mg/kg/day or 100 mg/kg/day) or propranolol (5 mg/kg/day or 25 mg/kg/day) administration for two weeks. Whereas GABA had no effect in control slices, it produced significant potentiation of NMDA after these pretreatments (*$p<0.05$).

receptor function was observed in the present work and similar results were seen with the theophylline pretreatment. A role of GABA receptor function in reducing essential tremor may be gleaned from previous studies (23). Harmaline-induced tremor is one of the well-studied experimental tremors (15). The tremor caused by harmaline as well as the associated increase of cyclic GMP in the cerebellum was antagonized by diazepam and ethanol at low doses (19). The inhibition by these compounds is thought to be mediated by GABA.

In the present work we have tried to evidence the unexpected beneficial effect of theophylline on essential tremor, an effect which is obtained at doses of this drug about ten times lower than usual for this xanthine. The side effects of theophylline in these twenty patients with essential tremor were negligible.

We have also sought the common changes in different receptor function assessed by modification of NMDA depolarisation after chronic propranolol and theophylline pretreatment. The increased sensitivity to GABA after both treatments might reflect a role for this inhibitory amino acid in reducing essential tremor.

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