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Influences of Immobilization and Footshock Stress on Pharmacokinetics of Theophylline and Caffeine in Rats

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Abstract

The influences of immobilization and footshock stress on pharmacokinetics of theophylline (20 mg kg^{-1}) and caffeine (30 mg kg^{-1}) administered orally were examined in rats.

The immobilization stress for 30 min or 1 h immediately after oral administration caused marked immobilization period-related decreases in plasma theophylline concentrations during the absorption phase, but did not affect plasma caffeine concentrations. The k_a and C_{max} values for theophylline were significantly decreased, and the t_{max} was significantly increased. On the other hand, when the immobilization stress was loaded for 1 or 3 h before the oral administration, the plasma theophylline or caffeine concentrations were not affected. The footshock stress for 30 min immediately after oral administration did not significantly decrease plasma theophylline concentrations during the absorption phase.

These results suggest that the pharmacokinetics of theophylline are influenced by strong stress, possibly due to the inhibition of its absorption from the gastrointestinal tract, but the pharmacokinetics of caffeine are not influenced by stress, probably due to its central action.

Recent studies have indicated that bronchial asthma is alleviated not only by standard medication but also by psychological therapy to exclude stresses (Teshima et al 1991; Irie et al 1992). Theophylline is widely used for the therapy of bronchial asthma, but the therapeutic window for the plasma concentration of theophylline is narrow, and the monitoring of the plasma concentration is often required in clinical treatment. It has been reported that the pharmacokinetics of theophylline are influenced by various factors such as age (Ogilvie 1978), smoking (Hunt et al 1976; Gomita et al 1991) and drug interactions (Mizuki et al 1989). However, there are only a few reports on the influence of stress on the pharmacokinetics of theophylline. In the present study, we examined the influence of immobilization and footshock stress on the pharmacokinetics of theophylline administered orally. In addition, the influence on the pharmacokinetics of caffeine was examined.

Materials and Methods

Animals

Male Wistar rats (Charles River Japan, Atsugi), 220–335 g, were housed in plastic-walled cages (26 × 36 × 25 cm), and food and water were freely available, except for 12 h before and during the experiment. The animals were maintained on a 12-h light–dark cycle (lights on from 0800 to 2000 h) at a room temperature of 22–24°C and a relative humidity of approximately 60%.

Drugs

Theophylline (Katayama Chemicals, Osaka, Japan) was suspended in 0·5% carmellose sodium solution and administered orally at 20 mg kg^{-1} (2 mL kg^{-1}). Caffeine (Nakarai Tesque Chemicals, Kyoto, Japan) was dissolved in distilled water and administered orally at 30 mg kg^{-1} (1 mL kg^{-1}).

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Determination of plasma concentrations of theophylline and caffeine

Blood samples were collected in a 60-μL capillary tube from the same part of the tail vein by cutting with a razor. After centrifugation at 11 500 rev min^{-1} for 3 min in a haematocrit centrifuge (Compur M 1100, Miles-Sankyo Co.), 20 μL plasma was added to 200 μL methanol containing 7-(2-hydroxyethyl)-theophylline (4 μg mL^{-1}) Tokyo Kasei, Japan as the internal standard and mixed for 30 s. After centrifugation at 10 000 rev min^{-1} for 5 min, 20 mL supernatant was injected into a high-performance liquid chromatography (HPLC) system. For the determination of caffeine, the concentration of the internal standard was decreased to 2 μg mL^{-1} and 40 μL supernatant was injected into the HPLC system. The HPLC system consisted of a pump (Hitachi Type 655-11), automatic sampler (Type 655A-40), processor (Type 655–61) and UV detector (Type 655A). A μBondapak C18 stainless-steel column (length, 300 mm; i.d., 3·9 mm; particle size, 10 μm, Waters Associates) was used at room temperature (21°C). The mobile phase was acetonitrile/0·01 M acetate buffer (pH 4·0) 1 : 12 (v/v) for theophylline and 1 : 10 (v/v) for caffeine. The flow rate was 1·5 mL min^{-1}. These compounds were detected at 280 nm (0·01 aufs). The retention times of theophylline and the internal standard were 8·1 and 10·1 min, respectively, and those of caffeine and the internal standard were 12·5 and 8·2 min, respectively. There were no interfering peaks in the chromatograms of plasma from control rats.

Experimental procedures

In the first experiment, immediately after oral administration of theophylline or caffeine, the rats were exposed to immobilization stress for 30 min or 1 h. In the second

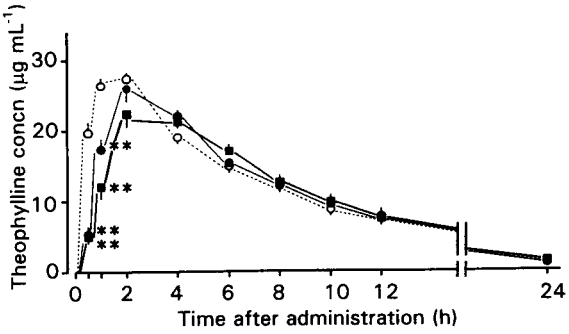

FIG. 1. Influence of immobilization stress on plasma theophylline concentrations in rats. Immobilization stress was applied for 0 (○), 30 min (●) or 1 h (■) immediately after oral administration of theophylline (20 mg kg^{-1}). Each value indicates the mean ± s.e.m. of six animals. $^{**}P < 0.01$ in comparison with the control group.

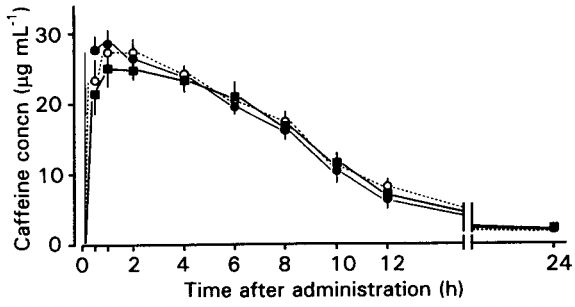

FIG. 2. Influence of immobilization stress on plasma caffeine concentrations in rats. Immobilization stress was applied for 0 (○), 30 min (●) or 1 h (■) immediately after oral administration of caffeine (30 mg kg^{-1}). Each value indicates the mean ± s.e.m. of 6–7 animals.

experiment, after the rats were exposed to immobilization stress for 1 or 3 h, theophylline or caffeine was administered orally. In the third experiment, immediately after theophylline or caffeine was administered orally, the rats were exposed to footshock stress for 30 min. In all experiments, the blood samples were collected repeatedly at various times between 30 min and 24 h after oral administration. Immobilization stress was employed by restraining animals in a flexible wire net. For the loading of footshock stress, a box (60 × 80 × 44 cm) with a floor consisting of 5-mm diam. stainless–steel rods spaced at 1-cm-intervals was used. The inside of the box was divided into 12 square compartments (20 × 20 cm) with white plastic walls. The box was equipped with tone and shock generators (Asteck Co., Japan). Rats were placed individually into each compartment, and monotone (2000 Hz) and footshock (1·8 mA) stimuli were applied. The monotone was delivered from 5 s before and during the footshock (10 s). This stimulus schedule was repeated at intervals of 90 s over 30 min (Yamori et al 1991).

Pharmacokinetic parameters

The absorption rate constant (k_a), the elimination rate constant (k_{el}), the volume of distribution (Vd), the time to reach the maximum concentration (t_{max}), maximum plasma concentration (C_{max}), the elimination half-life ($t_{\frac{1}{2}}$), the total clearance (CL) and the area under the plasma concentration–time curve (AUC_{0-24}) were estimated by non-linear least squares fit (MULTI) of data from each animal (Yamaoka et al 1981).

Statistics

Plasma concentration data and pharmacokinetic parameters were statistically analysed by analysis of variance followed by Duncan's test.

Results

Fig. 1 shows the effects of 30-min and 1-h immobilization stress after oral administration (20 mg kg^{-1}) on plasma theophylline concentrations. The plasma concentrations 30 min and 1 h after administration were significantly decreased by immobilization stress. Table 1 shows the pharmacokinetic parameters of theophylline in these experimental groups. The k_a and t_{max} values were significantly decreased and increased by immobilization stress, respectively, depending on the immobilization time. Although the C_{max} value was significantly decreased by 1-h immobilization stress, there were no significant differences in the $t_{\frac{1}{2}}$, CL and AUC values among the three groups. On the other hand, plasma caffeine concentrations after oral administration (30 mg kg^{-1}) were not affected by 30-min or 1-h immobilization stress after administration (Fig. 2).

When immobilization stress was given 1 or 3 h immediately before oral administration, the time courses of plasma

Table 1. Influence of immobilization stress on pharmacokinetic parameters of theophylline in rats.

Parameters	Control	Immobilization stress	
		30 min	1 h
K_a (h^{-1})	2·07 ± 0·30	1·32 ± 0·19*	0·75 ± 0·15**
K_{el} (h^{-1})	0·147 ± 0·01	0·153 ± 0·01	0·190 ± 0·03
Vd (L)	0·165 ± 0·01	0·165 ± 0·01	0·151 ± 0·02
t_{max} (h)	1·46 ± 0·15	2·32 ± 0·18**	3·00 ± 0·25**
C_{max} (μg mL^{-1})	27·52 ± 0·39	25·72 ± 1·13	23·07 ± 0·77**
$t_{\frac{1}{2}}$ (h)	4·82 ± 0·31	5·11 ± 0·48	4·49 ± 0·60
CL (L h^{-1})	0·401 ± 0·02	0·417 ± 0·04	0·434 ± 0·03
AUC_{0-24} (μg h mL^{-1})	244·95 ± 15·09	237·22 ± 17·10	230·37 ± 11·98

Theophylline was administered orally at a dose of 20 mg kg^{-1}. Immobilization stress was applied for 30 min or 1 h immediately after oral administration of theophylline. Each value indicates the mean ± s.e.m. of six animals. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with the control group.

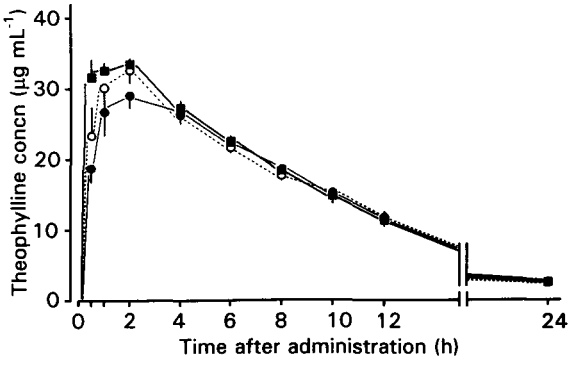

FIG. 3. Influence of immobilization stress on plasma theophylline concentrations in rats. Immobilization stress was applied for 0 (○), 1 (●) or 3 h (■) immediately before oral administration of theophylline (20 mg kg^{-1}). Each value indicates the mean ± s.e.m. of 5–6 animals.

theophylline concentrations showed no significant difference among the three groups (Fig. 3). The time courses of plasma caffeine concentrations were not significantly affected by such immobilization stress (data not shown). Fig. 4 shows the influences of footshock stress on plasma concentrations of theophylline and caffeine after oral administration. Footshock stress slightly decreased the theophylline concentrations during 0·5–1 h after administration, but there was no significant difference between the footshock and control groups. The plasma caffeine concentrations were not affected by footshock stress.

Discussion

The present results suggest that theophylline absorption from the gastrointestinal tract is impaired by immobilization stress, probably due to a reduction of gastrointestinal motility and mucosal blood flow. It has been reported that acoustic stress (Gué et al 1989), cold-restraint stress (Koo et al 1985) and wrap-restraint stress (Williams et al 1988) delay gastric emptying and decrease gastrointestinal motility. Although the influence of immobilization stress was marked during the absorption phase, rapid recovery of the plasma concentrations was observed after removal of the stress-exposure. In addition, when immobilization stress was applied for 1 or 3 h before oral administration of theophylline, the plasma concentrations were not affected. These results indicate that the pharmacokinetics of theophylline administered orally are influenced only during the period of stress-exposure. On the other hand, the pharmacokinetics of caffeine were not significantly influenced by immobilization stress either before or after the oral administration of caffeine. The stimulant effects of caffeine on the motor activity of mice and rats have been demonstrated by numerous studies. It is reported that the dose of caffeine to produce central nervous stimulant effect is 10–20 mg kg^{-1}, and spontaneous motor activity does not increase with doses above 30 mg kg^{-1} and even decreases with higher doses between 40 and 60 mg kg^{-1} (Nehlig et al 1992). Caffeine and theophylline significantly stimulate confinement motor activity during the period 30–45 min after the administration at an intraperitoneal dose of 20 mg kg^{-1} in rats (Thithapandha et al 1972). Furthermore, in drug distribution studies using microdialysis, the rate of penetration into brain extracellular space in rats is higher for caffeine than for theophylline when administered at a subcutaneous dose of 20 mg kg^{-1}. Caffeine is initially more rapidly distributed to the brain (the maximum concentration at 1 h after administration) than theophylline, and this finding may to some extent explain why caffeine, but not theophylline, is used as a central nervous system stimulant (Ståhle et al 1991). The lesser influence of immobilization stress on plasma caffeine concentrations than on theophylline concentrations may be due to the stimulant action of caffeine on the central nervous system, i.e. reducing the effect of immobilization stress.

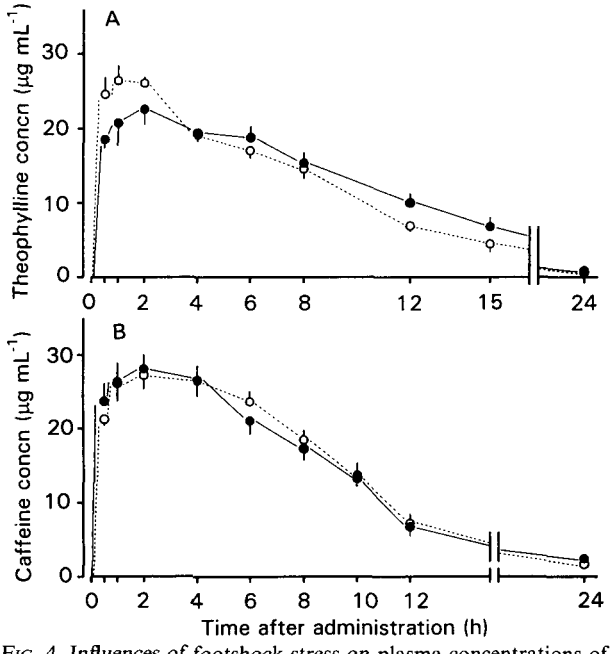

FIG. 4. Influences of footshock stress on plasma concentrations of theophylline (A) and caffeine (B) in rats. Footshock stress was applied for 0 (○) or 30 min (●) immediatedly after oral administration of theophylline (20 mg kg^{-1}) or caffeine (30 mg kg^{-1}). Each value indicates the mean ± s.e.m. of six animals.

Caffeine is absorbed more rapidly after oral administration than theophylline (Burg 1975). This was also confirmed in the present experiment, and might be another reason for lesser influence by immobilization stress on the absorption of caffeine than on theophylline. However, we previously showed that acute immobilization stress markedly decreased the plasma concentrations of nicorandil, which is absorbed more rapidly than caffeine (Yamori et al 1994). Further, we have also shown that footshock stress applied for 30 min immediately after the oral administration of nicorandil markedly decreased the plasma concentrations during both absorption phase and elimination phase (Yamori et al 1991). In the present study, however, the influence of footshock stress on plasma concentration of theophylline during elimination phase was not observed. The central action of theophylline may also attenuate the influence of footshock stress on plasma concentrations.

In conclusion, the present study shows that the pharmacokinetics of theophylline administered orally are influenced by stress-exposure, but the degree of the influence is comparatively weak, and the pharmacokinetics of caffeine is little affected by stress. Because methylxanthines are the

central nervous system stimulants, this action may be involved in these phenomena.

Acknowledgements

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