Effects of Caffeine and Theophylline on Activity of Rats in Relation to Brain Xanthine Concentrations[1] (36191)

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Despite numerous studies about the actions of caffeine and theophylline, little is known about the relationship between plasma and tissue levels of these xanthines and their effect in increasing motor activity of rats. This paper compares dose–response relationships for the stimulatory effects of these xanthines on motor activity in rats with xanthine concentrations in brain. We also report the rate of disappearance of caffeine and theophylline from plasma, muscle, and brain after intravenous administration and the binding of these xanthines to rat plasma proteins.

Methods. All experiments were performed on male Sprague-Dawley rats, which weighed 135–200 g. Confinement motor activity was measured in an apparatus similar to that described by Tedeschi *et al.* (1). The apparatus used in our study was constructed by the Biomedical Engineering Branch of NIH. The use of two racks, like that reported in a previous publication (2), permitted measurement of motor activity simultaneously in 12 rats, with each rat confined in a separate small compartment. Counts were recorded whenever a rat interrupted either of two photoelectric beams 8.5 cm above the floor of its compartment. The motor activity was measured for 90 min, with counts recorded every 15 min. Counts recorded for the first 15 min were disregarded because they showed considerable variability, probably reflecting the handling of the rats and their normal exploration of new surroundings. Solutions of caffeine and theophylline were prepared by dissolving the bases in saline, with warming, shortly before use. Caffeine, theophylline, or saline was injected ip in each rat immediately before it was placed in the apparatus for measuring confinement motor activity. The data obtained for different doses of the same xanthine were tested for significant differences by the Wilcoxon rank test [cited in Ref. (3)]. Means obtained for equal doses (mg of base/kg) of theophylline and caffeine were compared for significant differences by Student's t test. Differences were considered significant when $p < .05$.

In a separate experiment, body temperature was measured in rats with a Tele-Thermometer (Yellow Springs Instrument Co.). A piece of adhesive tape was placed around the probe for small animals, about 6 cm from the end. The probe was inserted into the rectum up to the piece of tape, thus insuring a constant depth of insertion from rat to rat. Each rat was housed in a separate small plastic cage (18 × 25 × 13 cm) during the experiment.

In experiments to determine the rate of disappearance from plasma and tissues, caffeine or theophylline (0.2 mmole/kg) was injected iv (tail vein). The animals were killed by decapitation at various times thereafter. Blood was collected in beakers which had been rinsed with a dilute solution of

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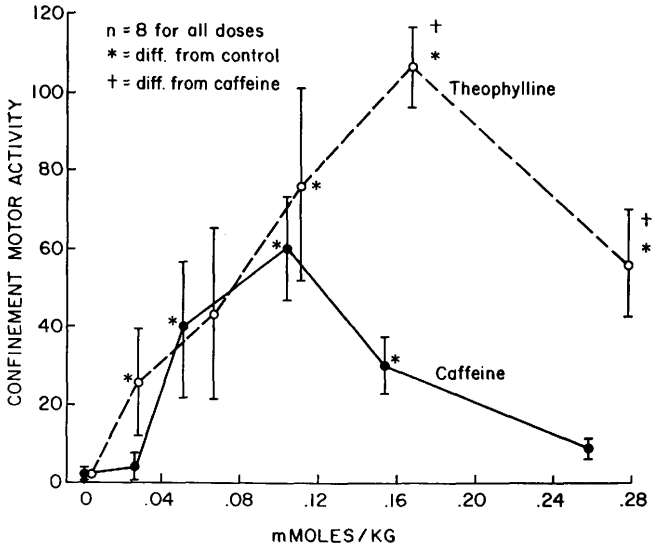

FIG. 1. Effects of caffeine and theophylline on confinement motor activity of male Sprague-Dawley rats: The values shown on the graphs represent the mean number of counts (± SE) recorded for 8 rats during the period 30–45 min after the administration of the xanthine. Caffeine, theophylline, or saline was injected ip in each rat immediately before it was placed in the apparatus for measuring confinement motor activity. The values indicated by asterisks are significantly greater than controls ($p < .05$). The daggers indicate that the values for theophylline are significantly greater ($p < .05$) than those for equal doses (mg of base/kg) of caffeine.

heparin (0.83 mg/ml) and dried. Thigh muscles and brains were rapidly removed, frozen in Dry Ice, and stored in a freezer for later analysis. One part by weight of brain or muscle was homogenized in an Omni-Mixer (Ivan Sorvall, Inc.) with 9 parts of phosphate buffer (0.067 *M*, pH 7.4), thus producing a 1:10 dilution. An aliquot (0.5–2.0 ml) of plasma or tissue homogenate was adjusted to a volume of 2.0 ml with the phosphate buffer. Theophylline was extracted into 20 ml of chloroform and caffeine into 20 ml of benzene. Measured volumes (15 ml) of the chloroform and benzene extracts were washed with 2 ml of phosphate buffer (saturated with sodium chloride). The xanthine was returned to the aqueous phase by shaking the organic phase with 1.0 ml of 4 *N* HCl. To remove traces of chloroform or benzene, the acid extract was washed with *n*-heptane (1.0 ml) before the optical density was read at 263 mμ for theophylline and 273 mμ for caffeine. Caffeine and theophylline have similar absorption curves, with a maximum at 263–264 mμ, as previously shown by Brodie *et al.* (4) and Axelrod and Reichenthal (5). The absorption of caffeine at 273 mμ, as used by Axelrod and Reichenthal (5), was more satisfactory for its determination than was 263 mμ because the blank was lower at 273 mμ. Since the optical density at 273 mμ in 4 *N* HCl in a quartz cuvette increases with time (5), the readings were taken within 2 min after the extraction of caffeine into the acid phase. These methods measure only the parent compounds, caffeine or theophylline, and none of their metabolites. Blanks are low (0.080–0.100). Drug concentrations as low as 1 μg/ml can be easily determined.

Plasma binding of either xanthine was measured by a modification of the method described by Toribara *et al.* (6). Visking tubing was soaked overnight in distilled water and blotted dry. About 15 cm of the tubing was cut off and tied with a double knot at one end. Plasma (2.0 ml) was equilibrated with 0.1 ml of caffeine or theophylline (10–1000 μg/ml) in a water bath (60°) for 5 min. The mixture was pipetted into the Visking bag which was suspended in a polyethylene centrifuge tube with the knotted end just touching the bottom of the tube. The open end of

TABLE I. Brain Levels (μmoles/g) of Caffeine and Theophylline After Intraperitoneal Injection of Equimolecular Doses (0.10 mmole/kg) in Rats.

Each value is the mean of the number of rats indicated in parentheses ± SE.

Drug	Minutes after drug		
	30	45	60
Caffeine	0.069 ± 0.006 (6)	0.063 ± 0.002 (3)	0.055 ± 0.003 (3)
Theophylline	0.074 ± 0.003 (3)	0.068 ± 0.002 (3)	0.058 ± 0.002 (3)

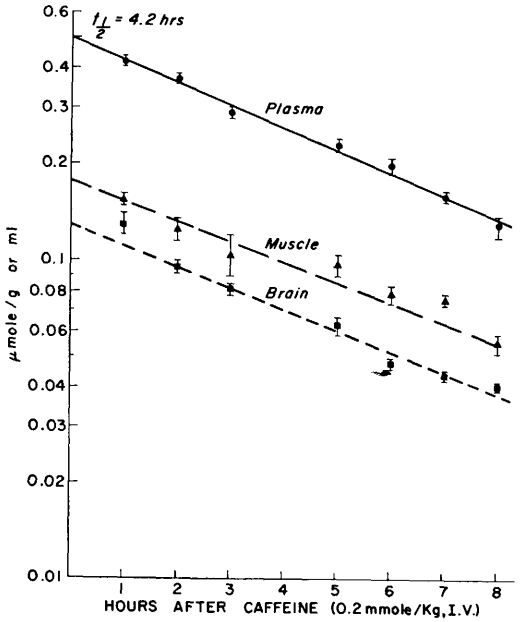

FIG. 2. Levels of caffeine in plasma, muscle, and brain after intravenous administration of the drug (0.2 mmole/kg) to rats: Each point represents the mean ± SE of measurements of tissue levels in three rats.

the bag was allowed to extend over the top of the centrifuge tube, and the bag was suspended in the tube by a rubber stopper. To eliminate the residual water in the Visking bag, the tube was centrifuged at 300*g* for 5–10 min, and the ultrafiltrate was discarded. Centrifugation at 300*g* for an additional 75–90 min then yielded an ultrafiltrate with a concentration of drug equal to the concentration of free drug in the bag. The amount of ultrafiltrate collected by this method was about 0.3–0.5 ml, an amount sufficient for assay of caffeine or theophylline. The percentage binding was calculated from the equation:

$$\% \text{ binding} = 100\ (C_0 - C_f)/C_0,$$

where C_0 is the initial concentration in the bag and C_f is the concentration of the ultrafiltrate.

Results and Discussion. When administered in doses equal or less than 0.12 mmole/kg, caffeine and theophylline increased confinement motor activity to about the same extent (Fig. 1). The peak motor activity after caffeine was produced by a dose of 0.10 mmole/kg (20 mg/kg). The maximum activity after theophylline occurred after a higher dose of 0.17 mmole/kg (30 mg/kg) and was appreciably greater than the maximal activity produced by caffeine. With doses of caffeine equal to or greater than 0.15 mmole/kg (30 mg/kg), motor activity decreased. Similarly, theophylline, in a dose of 0.28 mmole/kg (50 mg/kg), produced less activity than a dose of

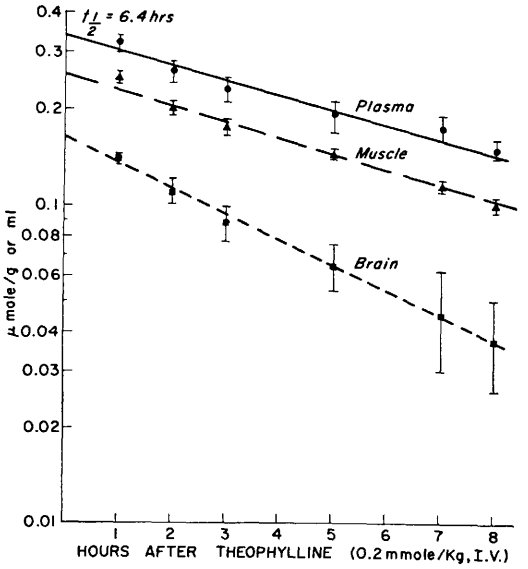

FIG. 3. Levels of theophylline in plasma, muscle, and brain after intravenous administration of the drug (0.2 mmole/kg) to rats: Each point represents the mean ± SE of measurements of tissue levels in three rats.

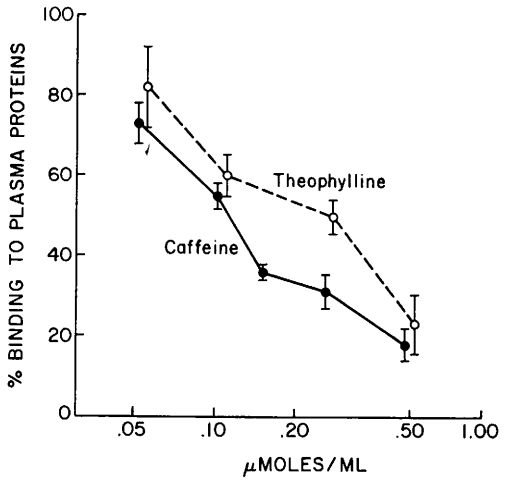

FIG. 4. Percentage binding of caffeine and theophylline to plasma proteins: Each point is the mean ± SE of three determinations.

0.17 mmole/kg (30 mg/kg).

After the administration of 0.1 mmole/kg, the brain levels of either xanthine were approximately constant, 0.055–0.075 μmole/g (10–14 μg/g), during the first hour (Table I). Measurement of motor activity of 3 rats in successive 15 min intervals indicated that the duration of response after a caffeine dose of 0.10 mmole/kg (20 mg/kg) varied between 75 and 180 min. When the stimulatory effect disappeared, the brain levels of caffeine were 0.015–0.026 μmole/g (3–5 μg/g). A larger dose of 0.26 mmole/kg (50 mg/kg) depressed motor activity initially and brain caffeine levels were higher (0.148 ± 0.009 μmole/g = 28.0 ± 1.7 μg/g in 3 rats 30 min after administration of the caffeine). This reduction of activity despite elevated brain levels of caffeine may reflect a toxic effect of the drug. The motor activity increased slowly with time as the brain level gradually declined. The brain levels of caffeine 150 min after the injection of this large dose (0.26 mmole/kg) in 3 rats were found to be 0.115 ± 0.006 μmole/g (22.3 ± 1.2 μg/g); at this time, the motor activity was increasing.

Figures 2 and 3 show the rate of disappearance of caffeine and theophylline from plasma, muscle, and brain after intravenous administration. The biologic half-life of caffeine in plasma was calculated as 4.2 hr (Fig. 2) and the corresponding half-life for theophylline was 6.4 hr (Fig. 3). The volume of distribution of caffeine was calculated to be about 0.4 liter/kg and that of theophylline about 0.6 liter/kg. Binding studies showed that both caffeine and theophylline at concentrations ranging between 0.05 and 0.60 μmole/ml were bound to plasma proteins as shown in Fig. 4.

The possibility was considered that the toxic effects of the higher doses of caffeine may be caused by an extreme elevation of body temperature. In a separate experiment, body temperature was measured in rats at

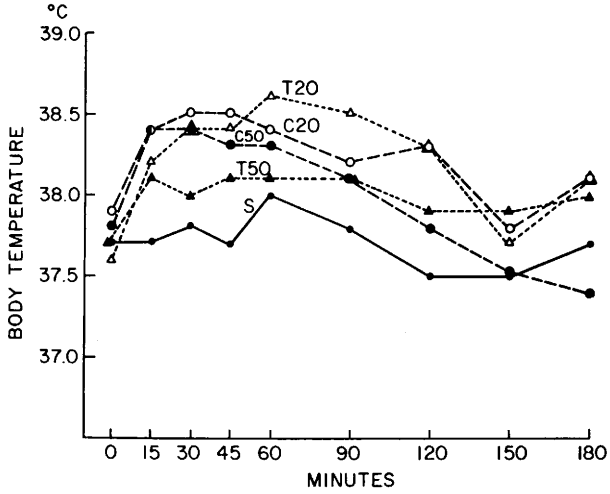

FIG. 5. Body temperature of rats during the first 180 min after ip injection (0 min) of saline (S); 20 mg/kg (0.10 mmole/kg) of caffeine (C20), 20 mg/kg (0.11 mmole/kg) of theophylline (T20); 50 mg/kg (0.26 mmole/kg) of caffeine (C50); or 50 mg/kg (0.28 mmole/kg) of theophylline (T50). Each value is the mean of measurements on 3 rats. SE values for rats which received saline were between 0.06 and 0.17°. SE values for treated animals were between 0.17 and 0.47°.

appropriate intervals for 3 hr after the administration of 20 and 50 mg/kg of caffeine (0.10 and 0.26 mmole/kg), and theophylline (0.11 and 0.28 mmole/kg). These doses of caffeine and theophylline affected body temperature only slightly (Fig. 5). The toxic effects of the larger doses of caffeine cannot be explained, therefore, on the basis of a drug-induced fever.

Summary. Caffeine and theophylline were equally effective in increasing confinement motor activity when administered ip in doses up to 0.10–0.12 mmole/kg. Higher doses of caffeine produced some toxicity as evident from less stimulation of motor activity. Theophylline produced its peak effect at a dose of 0.17 mmole/kg (30 mg/kg). Doses of either xanthine as great as 0.25 mmole/kg produced much less activity than the optimum doses. Equimolecular doses of caffeine and theophylline produced approximately equal brain xanthine levels.

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