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Caffeine disposition and effects in young and one-year-old rats

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Age often influences both the kinetics and toxicity of foreign compounds (Triggs & Nation 1975; Crooks et al 1976). One-year-old rats are more sensitive than younger ones to the lethal effects of caffeine (Peters & Boyd 1967). We have therefore studied the disposition of caffeine in 40-day and one-year-old rats after a single dose of 10 mg kg^{-1} to see whether there is a pharmacokinetic basis for the difference in toxicity.

We have also looked for biological determinants of the difference; this was done by using the liver perfusion technique (Bartošek et al 1973). We also wanted to establish whether any of the drug's biochemical effects, such as the increase in plasma free fatty acids (FFA) and corticosterone, were influenced by age. Two groups of male Sprague Dawley rats (Bio-Breeding Lab of Canada, Ottawa) 40-day and one-year-old, received 10 mg kg^{-1} of caffeine in water (1% w/v solution, NSDA†, Blended source) by gavage. Five animals were killed at each time (5, 15, 30, 60, 120, 240, 480 min) after administration. Blood was collected in tubes containing 0·1 ml EDTA (4%), centrifuged, and plasma was immediately frozen. Tissues were removed, rinsed in chilled 0·9% NaCl wiped and frozen. All tissues were homogenized in twice-distilled water (1 : 10 w/v).

The livers from four young and four old rats were isolated and perfused with recycling at 1 ml g^{-1} min^{-1} of flow following the technique of Bartošek et al (1973). Two doses of caffeine were used, a 10 mg kg^{-1} dose and ten times less in order to calculate kinetic parameters without any saturation of the eliminating systems that occur at higher concentrations (Aldridge et al 1977; Latini et al 1978). Microsamples (100 μl) were obtained from the circulating medium every 10 min for 180 min.

Caffeine was assayed by h.p.l.c. (Bonati et al 1979) in plasma and in some tissues at the time of plasma peak and 480 min after administration.

The concentration of caffeine vs time curves were analysed following a one-compartment open model system after extravascular administration. Experimental points were fitted by linear regression, after transformation by the peeling method. Apparent volume of distribution (Vd) and plasma clearance (Cl_p) were calculated from the following formulae:

$$Vd = \frac{F\,D}{AUC\,\beta}_{0\to\infty} \qquad Cl_p = \frac{F\,D}{AUC}_{0\to\infty}$$

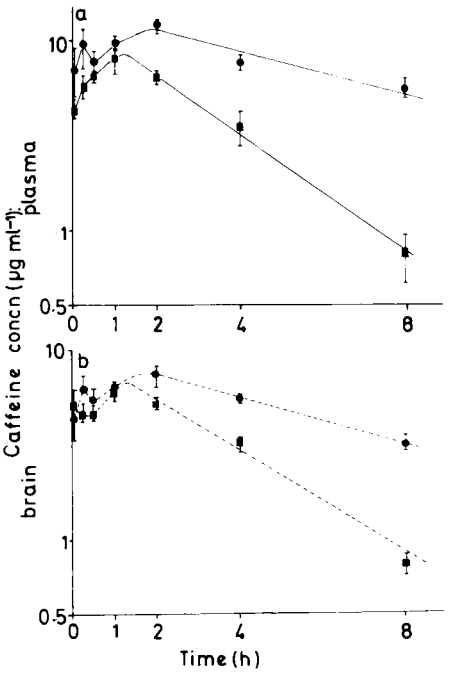

FIG. 1. a. Plasma caffeine concentrations in 40-day (■—■) and one-year-old rats (●—●) after 10 mg kg^{-1} by gavage. b. Brain caffeine concentrations in 40-day (■—■) and one-year-old rats (●—●) after 10 mg kg^{-1} by gavage. Vertical bars represent standard errors.

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† Kindly supplied by the National Soft Drink Association, Washington, U.S.A.

Table 1. Plasma and brain pharmacokinetic parameters of caffeine in 40-day and one-year-old male rats after acute administration.

Parameters	Plasma		Brain	
	40 days	1 year	40 days	1 year
Peak concn. (μg ml^{-1} (g^{-1}))	8·1 (60 min)	12·0 (120 min)	6·1 (60 min)	7·3 (120 min)
K_{abs} (K_t) (min^{-1})	0·26	0·012	0·009	0·013
$t_{\frac{1}{2}}$ (min)	120	332	134	300
AUC (μg ml^{-1} (g^{-1}) min^{-1}) $0\rightarrow\infty$	2061	6602	1752	3887
Vd (l kg^{-1})	0·806	0·714		
Clp (ml min^{-1} kg^{-1})	4·9	1·5		

Dose: 10 mg kg^{-1} in water (1% w/v solution) by gavage.
K_{abs} = rate constant of absorption.
K_t = rate constant of transfer to brain.
$t_{\frac{1}{2}}$ = apparent plasma (or brain) elimination half-life.
Cl_p = apparent plasma clearance.
AUC = area under plasma (or brain) conc. vs $0\rightarrow\infty$ time curve calculated with the trapezoidal rule with extrapolation to infinity.
Vd = apparent volume of distribution.

where: F is the bioavailable fraction, which was estimated to be equal to 1, since no caffeine was in the faeces of the treated rats (unpublished data); D is the administered dose (mg kg^{-1}); AUC $_{0\rightarrow\infty}$ is the area under the plasma concentration vs time curve calculated with the trapezoidal rule with extrapolation to infinity and β is the slope of the 'elimination' phase.

Experimental data from liver perfusions were treated according to the well-stirred model proposed by Pang & Rowland (1977). Plasma FFA were measured according to Trout et al (1960) and plasma corticosterone according to Guillemin et al (1959). Controls were killed at the same times as treated animals.

Plasma and brain concentrations of caffeine were higher in the old group at all times after administration (Fig. 1). Peak plasma concentrations occurred between 1 and 2 h in young but were delayed in old rats in which an early lower peak was seen at 15 min. The rate constant of absorption was also lower (0·012 min^{-1}) in old than in young rats (0·026 min^{-1}). Plasma apparent half-life was longer in old (332 min) than in young rats (120 min) and the area under the curve was larger in old (6602 μg ml^{-1} min^{-1}) than in young animals (2061 μg ml^{-1} min^{-1}) (Table 1).

Caffeine entered the brain rapidly and seemed to reach equilibrium within 5 min. The average brain: plasma ratio was 0·64 for old and 0·95 for young rats ($P<0·01$, Student's t-test for paired data). As shown in Table 2 caffeine was distributed to various organs with similar tissue: plasma ratios, except for adipose tissue where the ratio was much lower.

Average half-lives of the drug for young and old perfused livers were respectively 47 and 127 min for the low dose and 128 and 355 min for the high dose. Fig. 2 shows the difference between the two ages. Liver extraction ratios (E) were 0·09 for young and 0·03 for old livers (see Table 3).

Plasma FFA were significantly increased in one-year-old rats at 120, 240, 480 min (Table 4). Plasma corti-

Table 2. Caffeine concentrations (μg ml^{-1} or g^{-1}) in plasma, brain, heart, kidneys, adipose tissue and adrenal glands of 40-day and one-year-old male rats after acute administration.

	40 days		1 year	
Tissue	Plasma peak (60 min)	480 min	Plasma peak (120 min)	480 min
Plasma	9·22*† ± 0·41	0·75* ± 0·21	12·04 ± 1·22	5·50 ± 0·66
Brain	6·07 ± 0·74 (0·75)	0·72 ± 0·09 (0·96)	7·29 ± 0·95 (0·60)	3·16 ± 0·32 (0·57)
Heart	4·64 ± 0·65 (0·50)	0·35 ± 0·12 (0·47)	6·75 ± 0·88 (0·56)	3·81 ± 0·28 (0·69)
Kidneys	8·06 ± 0·98 (0·87)	0·59** ± 0·14 (0·79)	11·20 ± 1·32 (0·93)	6·06 ± 0·29 (1·10)
Adipose tissue	2·19 ± 0·23 (0·24)	n.m.	2·73 ± 0·26 (0·23)	1·63 ± 0·26 (0·30)
Adrenal glands	7·77 ± 0·95 (0·84)	n.m.	9·43+ ± 1·99 (0·78)	4·36 ± 0·87 (0·79)

Dose: 10 mg kg^{-1} of drug in water (1% w/v solution) by gavage.
* n = 4.
** n = 3.
† mean (n = 5) with s.e.
n.m.: not measurable (<0·02 μg g^{-1}).
( ): tissue: plasma ratio.

Table 3. Parameters for caffeine in perfused livers from young and old rats at 1 mg kg^{-1} dose (i.e. under linear conditions).

Age (months)	Dose (mg)	Volume of reservoir (ml)	$t_{\frac{1}{2}}$ (min)	C_0 (μg ml^{-1})	Vd (ml)	Cl (ml min^{-1})	Q (ml min^{-1} per liver)	E
1·3	93	93	42	·93	100	1·65	15·5	0·11
1·3	86	86	52	·99	87	1·16	14·3	0·08
12·0	106	106	126	·97	109	0·60	17·7	0·03
12·0	100	100	129	·95	105	0·56	16·7	0·03

$t_{\frac{1}{2}}$ = half-life of elimination.
C_0 = extrapolated concentration at time zero.
Vd = volume of distribution (dose/C_0).
Cl = total clearance (0·693 Vd/$t_{\frac{1}{2}}$).
Q = perfusion medium flow.
E = extraction ratio (Cl/Q).

costerone was significantly increased at 30 min in 40-day-old rats and at 60, 120, and 480 min in one-year-old rats (Table 5).

The strikingly longer half-life of caffeine can be explained by a lower clearance rate in one-year-old than in 40-day-old rats. Liver perfusion experiments showed this difference to be mainly owing to the hepatic disposition of the drug. This can be seen also from the ratios between half-lives of young and old rats which are 0·36 in vivo, and 0·37 and 0·36 in the perfused liver for the two doses. Since the extraction ratio is low, differences in hepatic blood flow depending on age are not relevant for clearance (Wilkinson & Shand 1975).

Caffeine is little bound to plasma proteins (10–20%) (Eichman et al 1962) so variations in the free fraction are not relevant for in vivo kinetics.

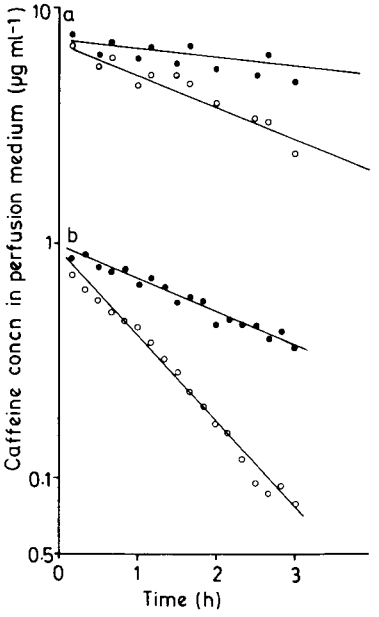

FIG. 2. Time course of disappearance from the perfusion medium of caffeine after 10 mg kg^{-1} (Fig. 2a) and after 1 mg kg^{-1} (Fig. 2b). Livers are from young (○—○) and old rats (●—●). Each point is the mean value of two experiments.

Moreover, in liver perfusion experiments we found the concentration of caffeine in the livers to be identical with that in the medium, so its intrinsic clearance is equal to the total clearance reported in Table 3 (Pang & Rowland 1977). This allows us to say that, even if elimination of caffeine is 'restrictive' (Wilkinson & Shand 1975), because of its low protein binding and low Cl/Q ratio (E), its extraction ratio should be essentially insensitive to changes in the free fraction of the drug. It may therefore be concluded that the differences in the enzymatic activity of hepatic parenchyma are probably the main determinants of the slower elimination rate of the drug in old rats.

The effect on plasma corticosterone and FFA concentrations seemed related in some way to the caffeine concentrations. Although other factors might be involved in these responses (adipose tissue mass, different rate of utilization of FFA; etc.), the different pharmacokinetic behaviour of young and old rats might be at least partially responsible for the different responses.

We have previously shown (Latini et al 1978), in agreement with Aldridge et al (1977), that at doses equal to or higher than 10 mg kg^{-1}, caffeine is eliminated in a dose-dependent way in the rat and mouse but not in the rabbit and in man (unpublished data). Present data suggest that age, as well as dosage, is another factor that can influence the response to caffeine. This might well

Table 4. Effect of caffeine on plasma FFA.

Time (min)	Plasma FFA (μmol litre^{-1}) 40 days Control	40 days Treated	1 year Control	1 year Treated
30	252±28	266±30	244±28	269±17
60	—	305±32	—	328±37
120	—	191±18	—	494±93*
240	312±40	292±22	210±20	488±22*
480	—	286±28	—	320±26*

Dose: 10 mg kg^{-1} of drug in water (1% w/v solution) (gavage).
Treated rats received 10 mg kg^{-1} of caffeine by gavage. Controls received saline and were killed at the times as treated animals.
* $P<0·05$ compared with controls.

Table 5. Effect of caffeine on plasma corticosterone.

Time (min)	Plasma corticosterone (μg/100 ml)			
	40 days		1 year	
	Control	Treated	Control	Treated
30	15±4	46±8*	10±2	15±1
60	—	23±4	—	55±6*
120	—	15±4	—	33±9*
240	21±5	29±1	19±3	lost
480	—	26±1	—	44±9*

Legend as in Table 4.

explain the data obtained by Peters & Boyd (1967) who found a significantly higher death rate in one-year-old rats than in 1·5–4·5-month-old rats given caffeine chronically at a daily dose of 185 mg kg^{-1}.

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Increase of cortical excitability induced by pentazocine

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Morphine (2 mg kg^{-1} i.v.) increases significantly the amplitude of the direct cortical response (Jurna et al 1972). This effect, interpreted as an increased excitability of cortical neurons, was recently supported by the results of electrophoretically administered morphine (Davies & Dray 1978).

To help elucidate whether this is an exclusive effect of morphine or a common characteristic of central analgesics, we have examined the action of pentazocine on the direct cortical response. This drug was selected because it is a central analgesic which antagonizes some actions of morphine (Acevedo et al 1967). Since naloxone has been reported as a narcotic antagonist (Martin 1976), its reversing effect on pentazocine actions was also investigated.

Materials and methods. Adult rats (n = 26), 200–250 g, under sodium pentobarbitone anaesthesia (50 mg kg^{-1} i.p.), (+)-tubocurarine and artificial respiration were used. The head was restrained in a Horsley-Clarke type stereotaxic apparatus and one cerebral hemicortex was exposed.

Direct cortical responses were elicited by supramaximal stimulation of the cortex with rectangular electrical pulses of 6 V, 0·01 ms duration and 0·25 Hz frequency applied by means of two silver ball electrodes, both being 2 mm from the recording electrode. The distance between stimulating electrodes was 1 mm.

The evoked potentials were recorded through a silver chloride electrode applied directly to the area SI of the cerebral cortex. The exact location of the electrodes was assessed by latency time analysis and testing fatigability of somatosensory evoked responses elicited by peripheral electrical stimulation. The responses were displayed on a Tektronix 502-A CRO and photographed with a Grass C4 camera. Measurements were made on the photographs.

The control group (n = 5) received 0·3 ml kg^{-1} of the solvent used for pentazocine (lactic acid 1·2% and NaCl 0·28% in distilled water). The experimental group (n = 21) received in 0·3 ml kg^{-1}, pentazocine at 10 mg kg^{-1} and 30 min later 11 animals of this group received in 0·5 ml kg^{-1}, naloxone at 0·5 mg kg^{-1}. Either the drugs or the solvent for pentazocine were administered by injection into the femoral vein. The mean amplitude of direct cortical responses was measured before solvent or drug injection so that each animal served as its own control. The amplitude variations of direct cortical responses obtained after solvent or drug injection were compared: (i) for solvent or pentazocine administration, with respect to the last value of the control period; and (ii) for naloxone

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