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Interactions of opioids with caffeine: evaluation by ambulatory activity in mice

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Abstract—Morphine (up to 10 mg kg^{-1}), buprenorphine (up to 0·1 mg kg^{-1}), pentazocine (30 mg kg^{-1}) and caffeine (up to 10 mg kg^{-1}), significantly increased mouse ambulation. The combination of morphine, buprenorphine and pentazocine with caffeine generally enhanced the effect. Dopamine D_1- and D_2-receptor bockade, depletion of stored dopamine, and inhibition of dopamine synthesis could reduce the ambulation increased by single administration of morphine, buprenorphine and caffeine, and by combined administration of morphine and buprenorphine with caffeine. Although naloxone (0·1–3 mg kg^{-1}) itself did not change mouse ambulation, at 3 mg kg^{-1}, it reduced the effect of caffeine. The repeated administration of morphine (10 mg kg^{-1}) induced a sensitization to the ambulation-increasing effect, and was inhibited by the combination of caffeine (10 mg kg^{-1}) in the repeated administration schedule. The repeated administration of caffeine (10 mg kg^{-1}) with buprenorphine (0·3 mg kg^{-1}) resulted in a decrease in the effect to the level of caffeine alone. The development of cross-sensitization to morphine (10 mg kg^{-1}) by the repeated treatment with buprenorphine (0·3 mg kg^{-1}) was inhibited by caffeine (10 mg kg^{-1}). Our results suggest that the dopaminergic systems are involved in the enhanced interaction of opioids having agonistic action on μ- or σ-receptors with caffeine. However, it is also considered that, following the repeated administration, caffeine acts to reduce the sensitivity to the ambulation-increasing effect of opioids, probably inducing up-regulation of adenosinergic systems.

Ambulation induced in mice by opioids is closely related to dopaminergic transmission through stimulation of opioid receptors, and the dopaminergic and opioid systems are involved in the reinforcing action, or dependence liability of opioids (Yanagita 1992). Repeated treatment with morphine elicits sensitization to the ambulation increase in mice (Kuribara & Tadokoro 1989), and it would be expected that this effect would be related to changes in the dopaminergic and opioid systems.

Caffeine is a CNS stimulant having weak reinforcing action (Deneau et al 1969) and is frequently contained in street narcotics, as an adulterant. It is, therefore, important to evaluate the interaction of caffeine with opioids.

Materials and methods

Animals. Male mice (dd strain, Institute of Experimental Animal Research, Gunma University School of Medicine, Japan) were housed in groups of 10 in standard aluminum cages (20 × 25 × 10 cm) under controlled conditions (temperature; 23 ± 2°C, r.h.; 50 ± 2%, 12 h light-dark schedule; light period of 0600–1800 h), on a solid diet (MF: Oriental Yeast, Tokyo, Japan) and tap water except during times of the experiment. Mice were used at 7 weeks old, 28–30 g.

Drugs. The drugs used were morphine HCl (Takeda Chemicals, Osaka, Japan), buprenorphine HCl (Lepetan Inj., Otuka Pharmaceutical, Tokyo, Japan), pentazocine (Pentagin Inj.; Sankyo, Tokyo), naloxone HCl (Sigma Chemical Co., St Louis, MO, USA), caffeine anhydrous (Kanto Chemical, Tokyo, Japan), SCH 23390 (*R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5,-tetrahydro-1H-3-benzazepine, Research Biochemical Natick, MA, USA), YM-09151-2 (*cis*-*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamine, Yamanouchi Pharmaceutical, Tokyo, Japan), reserpine (Apoplone Inj.; Daiichi Pharmaceutical, Tokyo), and α-methyl-*p*-tyrosine (AMPT, Sigma Chemical Co., St Louis, MO, USA). AMPT was suspended in saline containing a small amount of Tween 80 (2 drops per 5 mL) and administered intraperitoneally (i.p.). YM-09151-2 was first dissolved in a very small amount of 1 M HCl, and the solution was diluted with physiological saline. The other drugs were directly dissolved or diluted in the saline. These drugs were administered subcutaneously. The volume administered was 0·1 mL/10 g.

Apparatus and procedure. The apparatus for measurement of mouse ambulatory activity was a tilting-type ambulometer having 10 bucket-like plexiglass activity cages of 20 cm in diameter (SMA-10; O'Hara & Co., Tokyo, Japan). The apparatus recorded a slight tilt of the activity cage generated only by ambulation (locomotion) of the mouse, and not by pivoting or vertical movement such as rearing, sniffing or grooming.

Mice were individually placed into the activity cages, and after an adaptation period of 30 min, the drugs were administered. The ambulatory activity of each mouse was measured for 3 h.

Table 1. Mean 3-h ambulatory activity counts after subcutaneous administration of saline, caffeine, morphine, buprenorphine, pentazocine and naloxone alone, and combined administration of different doses of morphine, buprenorphine, pentazocine and naloxone with a fixed dose of caffeine (10 mg kg^{-1}).

Drugs	Doses (mg kg^{-1})	Activity counts (±s.e.m.) Single	Combined with caffeine
Saline		70 ± 8	
Caffeine	1	121 ± 20	
	3	105 ± 21	
	10	406 ± 39*	
	30	482 ± 48*	
Morphine	1	62 ± 10	649 ± 115[a]
	3	114 ± 27	1284 ± 246[a]
	10	1082 ± 174*	1580 ± 163[a]
	30	3387 ± 392*	3310 ± 405
Buprenorphine	0·03	137 ± 21*	684 ± 123[a]
	0·1	252 ± 80*	1288 ± 122[a]
	0·3	436 ± 80*	1384 ± 152[a]
	1·0	1054 ± 152*	1920 ± 300[a]
Pentazocine	1	83 ± 11	435 ± 65
	3	105 ± 19	421 ± 49
	10	127 ± 30	784 ± 131[a]
	30	980 ± 105*	572 ± 87[b]
Naloxone	0·1	71 ± 11	455 ± 79
	0·3	66 ± 8	297 ± 90
	1·0	75 ± 13	336 ± 50
	3·0	68 ± 8	290 ± 37[c]

In the combined administration, the drugs were administered simultaneously. * $P < 0.05$ vs the saline-administered value in the single administration schedule. [a], [b] and [c]: $P < 0.05$ vs the values after the single administration of both drugs, pentazocine alone and caffeine (10 mg kg^{-1}) alone, respectively (Dunnett's test).

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Table 2. Mean 3-h ambulatory activity counts after simultaneous subcutaneous administration of different doses of caffeine with a fixed dose of morphine (10 mg kg^{-1}), buprenorphine (0·3 mg kg^{-1}), pentazocine (10 mg kg^{-1}) or naloxone (1 mg kg^{-1}).

	Caffeine (mg kg^{-1})				
Drugs	0	1	3	10	30
Caffeine alone	70±8	121±20	105±21	406±39	482±48
Caffeine+morphine	1082±174	1128±224	1515±231	1580±183*	654±142†
Caffeine+buprenorphine	436±80	865±137*	823±143*	1384±152*	1152±125*
Caffeine+pentazocine	127±30	186±34	369±112*	784±131*	845±96*
Caffeine+naloxone	75±13	102±21	81±6	337±43	395±62

* and †: $P<0·05$ vs the values after the single administration of both drugs, and morphine alone, respectively (Dunnett's test). n=20 for the single administration, and n=10 for the combined administration.

Table 3. Effects of SCH 23390 (0·01 mg kg^{-1}, s.c.), YM-09151-2 (0·01 mg kg^{-1}, s.c.), reserpine (1 mg kg^{-1}, s.c.), and AMPT (200 mg kg^{-1}, i.p.) on the ambulation induced by single administration of morphine (10 mg kg^{-1}), buprenorphine (0·3 mg kg^{-1}) and caffeine (10 mg kg^{-1}), and combined administration of caffeine with morphine and buprenorphine.

Drugs	No treatment	SCH 23390	YM-09151-2	Reserpine	AMPT
Saline	73±10	38±7*	41±13*	3±1*	25±6*
Morphine	945±183	315±43*	215±53*	9±4*	261±99*
Buprenorphine	655±101	446±57*	204±41*	27±23*	97±33*
Caffeine	406±39	88±15*	140±38*	13±7*	121±19*
Caffeine+morphine	1353±149	1024±204	785±142*	66±32*	261±129*
Caffeine+buprenorphine	1297±202	551±101*	617±89*	293±169*	254±63*

SCH 23390 and YM-09151-2 were dosed simultaneously with, and reserpine and AMPT were dosed 4 h before, the administration of morphine, buprenorphine and caffeine. * $P<0·05$ vs the value without treatment with SCH 23390, YM-09151-2, reserpine and AMPT (Dunnett's test). n=10 in each experiment.

Table 4. Mean 3-h overall ambulatory activity counts after the repeated subcutaneous administration of morphine (10 mg kg^{-1}), buprenorphine (0·3 mg kg^{-1}), caffeine (10 mg kg^{-1}) alone, combination of caffeine with morphine and buprenorphine, and after challenge administration of morphine (10 mg kg^{-1}).

	Repeated administration					
Drugs	1st	2nd	3rd	4th	5th	Morphine
Saline	60±10	50±12	71±17	81±20	59±14	1046±138
Morphine	945±183	1676±296*	2115±457*	1669±439*	2165±558*	2339±579†
Buprenorphine	655±102	676±101	852±129	777±147	796±130	1759±158†
Caffeine	402±44	354±41	425±73	340±46	426±61	1043±137
Caffeine+morphine	1353±149	1606±188	1713±213	1584±158	1510±245	1086±223**
Caffeine+buprenorphine	1297±202	592±127*	451±119*	467±87*	419±75*	1120±181‡

The repeated administration was carried out at 3–4 day intervals, and the challenge administration was held four days after the 5th drug treatment. * $P<0·05$ vs the 1st administration within each group in the repeated administration schedule (Dunnett's test). †, ** and ‡: $P<0·05$ vs the saline, morphine and buprenorphine-alone experienced groups, respectively, in the challenge administration (Dunnett's test). n=10 in each group.

The experiments were held between 0900 and 1600 h. In each drug evaluation, 10–20 mice were used.

Statistical analysis. The mean overall ambulatory activity counts for 3 h were subject to analysis of variance. In the cases of significant overall variance, comparison between individual mean values was conducted using Dunnett's test. $P<0·05$ was considered significant.

Results and discussion

The results of the experiments are summarized in Tables 1–4.

Caffeine increased the mouse's ambulatory activity (Table 1). The present dose-effect relationship was similar to that previously reported (Fujii et al 1989). Caffeine has an antagonistic action on the adenosine receptor, and induces stimulation of dopaminergic systems through blockade of adenosine-related inhibitory systems (Cardinali 1980; Fredholm 1980; Snyder et al 1981).

Morphine, buprenorphine and pentazocine also increased the ambulatory activity (Table 1) with similar dose-effect relationships to those reported previously (Kuribara & Tadokoro 1989; Fujiwara et al 1990; Kuribara et al 1991). Morphine acts as a μ-receptor agonist, buprenorphine as a partial μ-receptor agonist-antagonist and κ-receptor agonist, and pentazocine as a μ-receptor antagonist and κ- and σ-receptor agonist (Cowan et al 1977; Tyers 1980; Jaffe & Martin 1985; Leander 1987). The ambulation increasing effect of opioids is considered to originate through agonistic action on the μ- or σ-receptors, and resulting acceleration of dopaminergic transmission (Rethy et al 1971; Buxbaum et al 1973; Kuschinski & Hornykiewicz 1974; Teitebaum et al 1979; Reggiani et al 1980; Iwamoto 1981; Swerdlow et

al 1985). Pentazocine has been reported to possess a direct action on dopamine receptors (Hernandez & Appel 1979, 1980). In agreement with these reports, the combination of morphine, buprenorphine and pentazocine with caffeine generally enhanced the ambulation-increasing effect (Table 1), suggesting the interaction of morphine, buprenorphine and pentazocine with caffeine produces acceleration of dopaminergic systems directly, or indirectly through agonistic action on μ- or σ-receptors, or antagonistic action on the adenosine receptor. On the other hand, the reduction of the ambulation-increasing effect following the combination of high doses of drugs, such as morphine (10 mg kg^{-1}) with caffeine (30 mg kg^{-1}) and pentazocine (10 mg kg^{-1}) with caffeine (10 mg kg^{-1}) may be due to non-specific behavioural disruption induced by over-doses (Table 2). This is because the mice given these drug combinations exhibited remarkable increase in behaviours indicating excitation, pivoting and rearing, which could not be recorded with our ambulometer.

The consideration that enhancement of the dopaminergic transmission is involved in the interaction of opioids with caffeine is supported by the blocking of the effect by SCH 23390 (dopamine D_1 antagonist, Iorio et al 1983; Mailman et al 1984), YM-09151-2 (dopamine D_2 antagonist, Terai et al 1983), reserpine (dopamine depleter) on AMPT (dopamine synthesis inhibitor) (Table 3). Particularly, the reduction of effect by reserpine was the strongest, indicating that release of stored dopamine is a principal process for the interaction of caffeine with morphine and buprenorphine. The doses of 0·01 mg kg^{-1} SCH 23390 and YM-09151-2 administered in this study were equipotent for suppression of many kinds of behaviour in mice (Kuribara & Uchihashi 1993); however, in the combination of morphine with caffeine, SCH 23390 did not significantly reduce the effect. This result suggests that the role of the D_1 receptor is smaller than that of the D_2 receptor in this drug combination.

Swerdlow et al (1985) reported that naloxone did not significantly modify the stimulant effect of caffeine in rats. In the present experiment, however, the highest dose of naloxone (3 mg kg^{-1}) significantly reduced the effect of caffeine. According to our experience, up to 2 mg kg^{-1} naloxone could reduce the ambulation-increasing effect of methamphetamine and cocaine (Kuribara unpublished data), suggesting that naloxone acts to reduce dopaminergic transmission, and that this mechanism is involved in the reduction of the effect of caffeine by naloxone.

The repeated administration of buprenorphine alone produced no significant change in individual effects (Table 4). These results are consistent with our previous report (Kuribara et al 1991). The repeated administration of morphine induced sensitization to its ambulation-increasing effect. The agonistic action on κ-receptors is considered to suppress the induction of sensitization to the ambulation-increasing effect through μ-receptors (Kuribara et al 1991).

The repeated administration of caffeine alone did not change its ambulation-increasing effect (Table 4). It has also been demonstrated that repeated treatment with caffeine did not change the stimulant actions of caffeine itself and methamphetamine (Fujii et al 1989). However, the present experiment demonstrated that caffeine acted to inhibit the development of morphine sensitization, to reduce the effect of buprenorphine and to inhibit the cross-sensitization from buprenorphine to morphine. Repeated treatment with caffeine induces up-regulation of the adenosine receptors and results in tolerance to its stimulant effect (Holtzman 1983; Chou et al 1985; Holtzman et al 1991). It is possible that such caffeine-induced changes in the adenosinergic systems may be involved in the inhibition of the morphine- and buprenorphine-induced acceleration of dopaminergic transmission; further study is required to elucidate the mechanism.

The behavioural study of the interactions of opioids with caffeine is important for assessment of the problems of combined abuse of these drugs. The enhancement of the ambulation-increasing effect following the combined administration may be relevant to the presence of caffeine in some street narcotics (particularly heroin in Japan), probably to enhance the reinforcing action. Moreover, the inhibition by caffeine of the induction of morphine sensitization is considered to be relevant to an increment of the dose of narcotics following the repeated abuse, resulting in an increased probability of the intoxication to narcotics after the administration of an over-dose.

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Effects of hypolipidaemics cetaben and clofibrate on mitochondrial and peroxisomal enzymes of rat liver

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Abstract—Clofibrate or cetaben was administered to male rats for 10 days. Peroxisomal and mitochondrial enzymes were assayed in liver subcellular fractions. Clofibrate affected the specific activities of both mitochondrial enzymes (glycerol-3-phosphate dehydrogenase and nicotinamide-linked isocitrate dehydrogenase) and peroxisomal enzymes (fatty acyl-CoA oxidase, glycerone phosphate acyltransferase, urate oxidase, and D-amino-acid oxidase). In contrast, cetaben raised only the peroxisomal enzymes, acyl-CoA oxidase, glycerone-phosphate acyltransferase, D-amino-acid oxidase, catalase, and urate oxidase. Thus, the hypolipidaemic activity of these drugs may be exclusively related to stimulated peroxisomal functioning, while mitochondria play only a minor role.

Although only a few mitochondrial enzymes have been shown to be influenced by clofibrate, mitochondria have received particular interest in studies on the hypolipidaemic action of the drug. Mitochondrial enzymes which are induced by clofibrate include glycerol-3-phosphate dehydrogenase (Hess et al 1965), choline dehydrogenase (Kramar et al 1984), outer carnitine palmitoyltransferase (Markwell et al 1977), and nicotinamide dependent isocitrate dehydrogenase (Schön et al 1991).

Cetaben has been identified as an anti-atherosclerotic hypolipidaemic substance (Hollander et al 1978; Fort et al 1983). It is structurally quite different from clofibric acid but is also an amphipathic carboxylate. Hypolipidaemic peroxisome proliferators often have this structural feature. Cetaben has been shown to act similarly in some respects to clofibrate. In rats, cetaben treatment at a dose of 200 mg kg^{-1} day^{-1} raised liver weight slightly and the number of peroxisomes increased, as did liver catalase activity (Fort et al 1983).

The effect of cetaben on mitochondrial and other peroxisomal enzymes has not been investigated so far; we have, therefore, compared the action of cetaben with that of clofibrate by measuring the activities of selected mitochondrial and peroxisomal enzymes in rat liver cell fractions.

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Materials and methods

Materials. Cetaben was a generous gift from Lederle Arzneimittel GmbH & Co. (Wolfratshausen, Germany). Clofibrate was purchased from Serva (Heidelberg, Germany). D-[U-^{14}C] Fructose 1,6-bisphosphate (sp. act. 12·5 MBq mM^{-1}) was purchased from Amersham (Buckinghamshire, UK). Triose-phosphate isomerase (5 k units mg^{-1}) and fructose-bisphosphate aldolase (9 units mg^{-1}) were obtained from Boehringer Mannheim GmbH (Germany). All other reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Animals and treatment. Male Sprague-Dawley rats, 181 ± 25 g, were allowed free access for 10 days to a standard laboratory chow (controls), or one containing either 1·0 g kg^{-1} cetaben or 7·5 g kg^{-1} clofibrate. Food for the drug treatment was impregnated with cetaben dissolved in chloroform/methanol (3:1) or with clofibrate dissolved in diethylether. Solvents were removed by evaporation to dryness. The mean daily intake of clofibrate was 840 mg kg^{-1} day^{-1} and that of cetaben 112 mg kg^{-1} day^{-1} calculated from the daily food consumption of 20·2 g. The cetaben dose was chosen after preliminary experiments: 56 mg kg^{-1} day^{-1} did not lower the plasma triglycerides significantly, whereas 335 mg kg^{-1} day^{-1} caused the death of three out of six rats during a feeding period of 10 days. A comparable toxicity of cetaben has been reported (Oker-Blom 1981). The clofibrate dose was within the range used in other experimental studies on rat liver (Hess et al 1965; Kurup et al 1970). No animal died under this regimen. This was also the case in our extensive animal studies on clofibrate (Kramar et al 1984; Schön et al 1991; Prager et al 1993). The 10-day feeding period was adopted in accordance with the literature (Hess et al 1965).