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Serum and brain concentrations of pentazocine in relation to analgesic activity in mice

LENNART PAALZOW AND ASTRID ARBIN

Apotekens Centrallaboratorium, Box 3045, *S*-171 03 *Solna, Sweden*

Serum and brain concentrations of pentazocine and its metabolite, the *trans*-alcohol, have been studied in mice and related to analgesic activity. The concentrations were determined fluorometrically after extraction and isolation of the compounds by partition chromatography as ion pairs. The acute intravenous toxicity (LD50) of the drug was also determined. The analgesic activity was found to reside in the unchanged pentazocine since, after the initial distribution phase, the drug elicits a higher degree of analgesia in animals showing higher brain concentrations of pentazocine. Furthermore, animals which did not show an analgesic response had higher serum concentrations of the *trans*-alcohol in comparison with animals showing analgesic activity, indicating a relation between analgesia and the degree of metabolism. However, the relation between the analgesic response and the brain concentration during the first 20 min after intravenous injection of the drug was poor.

The dose-response relation of the analgesic activity of morphine is related to its brain concentration, but there is a poor time-course relation between activity and drug concentration (Paalzow & Paalzow, 1971a). Subsequently the relations between morphine-induced analgesia and brain catecholamines were examined (Paalzow & Paalzow, 1971b), and now further studies on the mechanism of action of analgesic drugs, with pentazocine as the test substance, are described.

Pentazocine possesses both agonistic and antagonistic properties. It is a relatively weak antagonist and it has been shown to be about as effective an agonist as morphine in man (Fraser & Rosenberg, 1964; Jasinski, Martin & Hoeldtke, 1970). In animals, the activity varies according to the parameter measured (Hoffmeister & Kroneberg, 1965; Paalzow, 1969b; Gray, Osterberg & Scuto, 1970).

Pentazocine is metabolized firstly to two isomeric alcohols, and then to the corresponding carboxylic acid (Pittman, Cherniak & others, 1969). These authors found the *trans*-alcohol (OH-pentazocine) to be the primary metabolite in extracts of mouse liver. The relations between the analgesic activity and serum and brain concentrations of this metabolite and unmetabolized pentazocine are now described.

MATERIALS AND METHODS

Male albino mice of the N.M.R.I. strain, 17–23 g, were allowed free access to water for 16 h before the test, but no food. (±)-Pentazocine lactate was administered intravenously and the dose was calculated as the free base. Physiological saline solution was used as control.

Electrical stimulation was by square waves from two stimulators (*Grass Instruments S4*).

The analgesic activity was determined according to Paalzow (1969a, b). The tail

of the mouse is stimulated for 40 ms, at 125 Hz and a pulse-width of 1·6 ms. The electrodes (injection needles No 20) are placed 20 mm apart intracutaneously in the middle section of the tail with the positive pole in the proximal position, and are not removed during the experiment.

A nocicentive response is assumed to be produced when one of the first three consecutive shocks applied with an interval of 1 s produces vocalization. The threshold for each animal is determined before administration of the drug by increasing voltage logarithmically from 4 V until a response is obtained. After injection of the drug, the threshold voltage was tested at 10 min intervals for 1 h. The response was graded and expressed as a percentage of the pretreatment threshold voltage.

Pentazocine and its metabolite, the *trans*-alcohol (OH-pentazocine), were determined in serum and brain tissues after extraction as ion pairs with perchlorate, and subsequently isolated by partition chromatography as ion pairs with chloride according to Borg & Mikaelsson (1970). Serum (0·2 ml) and M $HClO_4$ (0·2 ml) were homogenized with 0·5 g cellulose and packed into a column. Pentazocine and OH-pentazocine were then extracted with dichloromethane (5 ml), and the extract collected in a silanized centrifuge tube with tapered bottom. 1-Pentanol (25 μl) was added and the dichloromethane phase was evaporated to a final volume of about 50 μl. The remaining solution was then quantitatively injected into a separation column for isolation of pentazocine and OH-pentazocine. Ethanolized cellulose was used as support and N HCl as the stationery phase. The mobile phase was cyclohexane–1-pentanol (8:2). The column was eluated at a rate of 0·5—1 ml/min. The pentazocine (4–7 ml) and OH-pentazocine (25–35 ml) fractions were collected, extracted into 1·00 ml 0·1 N phosphoric acid and measured fluorometrically at 278/324 nm. The concentrations were evaluated by standard curves. Brain concentrations of pentazocine and OH-pentazocine were determined in the same way as for serum, except that each single brain was homogenized twice with a total of 3 ml M $HClO_4$, after which it was mixed with 3 g cellulose, and subsequently extracted with 20 ml dichloromethane. The acute toxicity of pentazocine (LD50) was determined according to Pharmacopoeia Nord. IV (1960).

RESULTS AND DISCUSSION

Analgesic activity. Pentazocine was reported to have an ED50 of 7·4 (5·9–9·6) mg/kg intravenously in the present test by Paalzow (1969b). A dose 10 mg/kg intravenously was used in the present study, Maximal activity was found 30 min after injection with an average increase in threshold of about 100% (Fig. 1). The same time lag for maximal effect was found in rats after 15 mg/kg pentazocine given subcutaneously (Berkowitz & Way, 1971). Morphine given orally or subcutaneously showed the same time lag for maximal effects, as when administered intravenously (Paalzow, 1969b).

Acute toxicity. The intravenous LD50 determined at three dose levels each with 15 mice was 26·7 mg/kg, with confidence limits of 24·2–28·9 mg/kg at $P = 0{\cdot}05$. In most animals death occured in 15 min; all animals survived a dose of 20 mg/kg. The dose used in the analgesic experiments was thus well below the LD50 dose.

Serum and brain concentration. The serum concentrations after pentazocine (10 mg/kg, i.v.) declined rapidly but after 20 min the rate of decay slowed (Fig. 2A). The same time-course for the disappearance of the drug (10 mg/kg, i.v.) has been found in rats by El-Mazati & Way (1971). Pentazocine rapidly reached to the

brain and gave concentrations higher than those in serum (Fig. 2B). During the first 20 min, the brain concentrations were about 3·5–6 times higher than those in serum, and 40 min after administration this ratio had decreased to 2; 40 min after administration, less than 20% of the concentration at 5 min was present. These findings are in accordance with those found in rats (El-Mazati & Way, 1971; Medzihradsky & Ahmad, 1971). In the toxicity study, most animals died during the first 15 min after injection; this corresponds to the highest concentration of pentazocine in serum and brain.

The relation between analgesic activity and serum and brain concentrations of pentazocine and OH-pentazocine. The results described suggest the relation between the analgesic activity and the serum and brain concentrations of drug during the

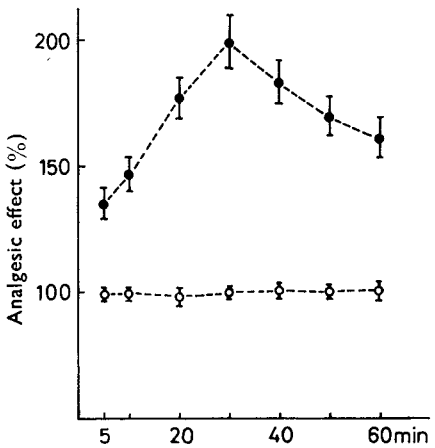

FIG. 1. The analgesic activity in mice after intravenous administration of 10 mg/kg pentazocine. Each point represents the mean ± s.e. of 200 animals given pentazocine (●) and 40 animals given placebo (○).

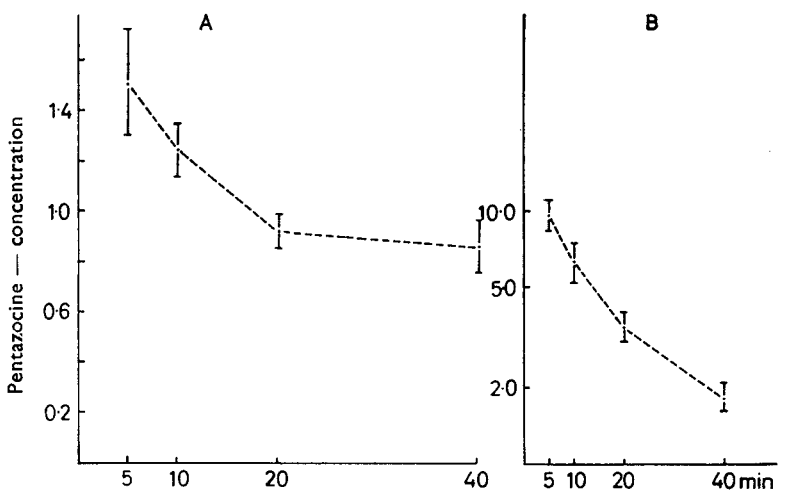

FIG. 2.A. Serum concentrations of pentazocine (μg/ml) in mice after intravenous administration of 10 mg/kg pentazocine. Each point represents the mean ± s.e. of 10 animals.

B. Brain concentrations of pentazocine (μg/g) in mice after intravenous administration of 10 mg/kg pentazocine. Each point represents the mean ± s.e. of 17 animals.

first 20 min. to be poor; the brain concentrations fall while the activity rises. Forty min after injection both show a decreasing trend.

In subsequent experiments, this relation was further investigated by examining individual differences in analgesic activity in the animals. These experiments were performed in parallel with analyses of the serum and brain concentrations of pentazozine and OH-pentazocine.

After intravenous injection of 10 mg/kg pentazocine, the animals were tested at fixed intervals for analgesic activity, and killed immediately after the test and arbitrarily divided into two groups: one in which analgesic activity was found and one in which it was absent. The results of the serum investigation can be seen in Table 1.

Table 1. *Serum concentration of pentazocine and OH-pentazocine after intravenous administration of 10 mg/kg pentazocine.* The animals were divided into two groups with different degrees of analgesia, and killed 5, 20 and 40 min after injection.

Number of animals	Time after injection (min)	Analgesia % threshold value ±s.e.	*P*	Serum pentazocine μg/ml ±s.e.	*P*	Serum OH-pentazocine μg/ml ±s.e.	*P*
5	5	179 ± 14	<0·001	1·96 ± 0·30	<0·05	1·66 ± 0·69	>0·05
5	5	104 ± 4		0·81 ± 0·12		3·38 ± 1·78	
4	20	132 ± 10	>0·05	1·07 ± 0·03	>0·05	1·50 ± 0·77	>0·05
8	20	105 ± 5		1·65 ± 0·63		1·07 ± 0·22	
5	40	235 ± 45	<0·05	1·01 ± 0·07	<0·01	0·36 ± 0·16	<0·01
5	40	105 ± 5		0·46 ± 0·10		0·73 ± 0·10	

Mice with significant ($P < 0·05$) analgesic activity had significantly higher sèrum concentrations of pentazocine than animals showing no effect. The average concentration from the total material decreased with time, however (Fig. 2B). Mice not showing analgesia had higher serum concentrations OH-pentazocine compared with animals showing activity, and this difference was significant 40 min after administration. Thus, mice lacking effect seem to metabolize pentazocine more rapidly and extensively than animals showing analgesic effect. At a sensitivity limit of 100 ng/g for chemical analysis, no OH-pentazocine could be detected in brain tissues.

The brain concentrations of pentazocine obtained similarly to those for serum can be seen in Table 2. At 10–40 min after injection (10 mg/kg, i.v.) animals with significant analgesic activity had significantly higher brain concentrations of pentazocine than animals showing no effect; the brain concentrations decreased with time (cf. Fig. 2B).

These experiments suggest that pentazocine is active *per se* since, after the initial distribution phase, it gives a higher degree of analgesia in animals exhibiting a higher concentration in brain. Furthermore, Berkowitz & Way (1971) reported that, of the known metabolites of pentazocine, all are practically without analgesic activity compared with pentazocine (information from C. Davison, Sterling-Winthrop).

Our present investigations were thus confirmed, since mice lacking analgesia seem to metabolize pentazocine to a higher degree than animals showing an effect. On the basis of earlier estimations of the analgesic activity of pentazocine, Paalzow (1969b) proposed that a rapid or a high degree of biotransformation in mice could explain why this drug had failed to be effective or had shown a limited activity in some

Table 2. *Brain concentration of pentazocine after intravenous administration of 10 mg/kg pentazocine.* The animals were divided into two groups with different degrees of analgesia and killed 5, 10, 20 and 40 min after injection.

Time after injection (min)	Number of animals	Analgesia % threshold value ± s.e.	*P*	Pentazocine concn in brain μg/g ± s.e.	*P*
5	5	151 ± 13	<0·01	11·78 ± 1·35	>0·05
5	11	88 ± 3		8·19 ± 1·79	
10	8	291 ± 46	<0·001	8·75 ± 1·72	<0·01
10	8	94 ± 5		3·38 ± 0·52	
20	8	290 ± 56	<0·001	3·87 ± 0·42	<0·05
20	6	104 ± 7		2·41 ± 0·42	
40	8	213 ± 26	<0·001	2·26 ± 0·30	<0·01
40	6	90 ± 9		0·99 ± 0·15	

animal tests. The mouse seems to metabolize pentazocine to a different extent and in a different manner than man. In a mouse liver preparation the *trans*-alcohol was primary metabolite (Pittman & others, 1969). In human urine, however, Pittman (1970) found the *cis*-alcohol and the corresponding carboxylic acid to be major metabolites of pentazocine.

During the first 20 min after injection, we found the relation between the brain concentration and the degree of analgesia to be poor. This can be explained by a relatively slow distribution within the brain or by pentazocine inducing some biochemical changes in the brain which elicit analgesia. The same conclusions were drawn from experiments with morphine (Paalzow & Paalzow, 1971a).

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