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SITE OF ANALGESIC ACTION OF A NON-STEROIDAL, ANTI-INFLAMMATORY DRUG, TOLMETIN SODIUM, IN RATS

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1 The site of the analgesic action of tolmetin sodium was investigated by use of the acetic acid writhing test in rats.

2 Tolmetin sodium was administered to the rat between 15 and 60 min after an intraperitoneal injection of 1 ml of a 1% acetic acid aqueous solution. Number of writhings was counted for 20 min beginning from 60 min after acetic acid injection.

3 When the rat was given tolmetin sodium 5 mg/kg orally, a relatively large quantity of tolmetin was found in the peritoneal exudate and there was a rough correlation between anti-writhing activity and the exudate tolmetin content.

4 Anti-writhing ED_{50} of tolmetin sodium was 1.42 (0.82–2.91) and 92.0 (57.0–140) μg/kg when given intraperitoneally and intravenously, respectively, and the potency ratio of intraperitoneal to intravenous tolmetin sodium was 40.0 (18.5–80.2). This potency ratio for salicylic acid and morphine hydrochloride was 19.4 and 1.0, respectively.

5 When equipotent doses (5 μg/kg i.p.; 200 μg/kg i.v.) of tolmetin sodium were administered to the rat, the plasma tolmetin level after the intraperitoneal administration was less than one-fortieth that after the intravenous administration during the counting time of 20 min, while both the peritoneal exudate contents of tometin were nearly equal.

6 From these results, it is concluded that the site of anti-writhing action of tolmetin sodium is in the peritoneum and that tolmetin sodium produces its anti-writhing action mainly by a peripheral mechanism in the rat.

Introduction

Tolmetin sodium, a non-steroidal anti-inflammatory agent being a pyrrole acetic acid derivative, has been reported to have considerable analgesic and anti-inflammatory efficacy in man (Berkowitz, 1974; Mainbach, 1976; Bachman, Stroescu & Hartl, 1977). In experimental animals, it has been reported that tolmetin sodium has relatively more potent analgesic (anti-writhing) activity than anti-inflammatory activity, while it shows inhibitory activities on many kinds of experimentally induced inflammations (Wong, Gardocki & Pruss, 1973; Shimizu, Nakamura, Motoyoshi & Yokoyama, 1975; Nakamura, Yokoyama, Motoyoshi, Ishii & Shimizu, 1979a; Nakamura, Ishii, Motoyoshi, Imazu, Yokoyama & Shimizu, 1979b). Anti-writhing activity of tolmetin sodium has been reported to be 10 times or more that of aspirin and 2 to 10 times that of ibuprofen in the mouse writhing tests, using phenylquinone, acetylcholine and acetic acid as algesic chemicals (Wong *et al.*, 1973; Shimizu *et al.*, 1975; Nakamura *et al.*, 1979b), and to be about 8 and about 4 times as potent as aspirin and ibuprofen, respectively, in the rat acetic acid-writhing test (Nakamura *et al.*, 1979b). Tolmetin sodium, however, does not show analgesic activity in the tail flick or Haffner tests, and its anti-writhing action is not reversed by naloxone, nor is it influenced by repeated administration (Nakamura, Ishii, Yokoyama, Motoyoshi, Imazu & Shimizu, 1977). These findings suggest that tolmetin sodium, unlike morphine, produces its analgesic action by a peripheral mechanism. This supposition concerning tolmetin sodium has not been confirmed and the site of analgesic action of most non-steroidal anti-inflammatory agents is not clear. This investigation was therefore carried out to determine the site of the analgesic action of tolmetin sodium in the acetic acid-writhing test in rats.

Methods

Analgesic assay

Male, Wistar rats weighing 100 to 110 g were used. Each rat was given intraperitoneally 1 ml of a 1% acetic acid aqueous solution, and was settled singly into a cylindrical cage (24.5 cm in diameter) made of transparent acrylic resin. Only rats showing the wri-

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thing syndrome within 15 min after acetic acid injection, were used for the experiment. Drugs were administered to the rats between 15 to 60 min after the acetic acid injection and then the number of writhings was counted for 20 min, beginning at 60 min after the acetic acid injection (Niemegeers, Van Bruggen & Janssen, 1975; Nakamura *et al.*, 1979b).

Determination of tolmetin in plasma and exudate

The blood was obtained by heart puncture under ether anaesthesia from the rat receiving acetic acid (i.p.) and then the exudate in the peritoneal cavity was washed out with 10 ml of distilled water. Tolmetin in plasma and exudate was extracted and determined according to the procedure of Kojima & Nakanishi (unpublished) as follows: 1 ml of plasma was acidified with 2 ml of 1 N HCl, shaken with 5 ml of ethylether for 15 min and then centrifuged at 3,000 rev/min for 15 min. Four ml of the ether layer was evaporated by bubbling with air, and the residue was dissolved in 0.2–0.4 ml of methanol. The exudate with washings was acidified with 3 ml of 1 N HCl, shaken with 15 ml ethylether for 15 min, and centrifuged for 15 min. Ten ml of the ether layer was evaporated and the residue dissolved in 0.2–0.4 ml of methanol. Twenty μl of the methanol solution was injected into a column for high-performance liquid chromatography (HPLC).

A Waters Model 204-chromatography and μ-Bondapak NH_2 column were used; Model 6000A Solvent Delivery System, Model U6K Universal Injector, and Model UV440 Absorbance Detector. The eluant consisted of a mixture of 1% acetic acid, methanol and acetonitrile (5/2/16 by vol). The flow rate was 2.5 ml/min and the effluent was monitored at 254 nm. Retention time of tolmetin is 2 min. The lower limit of detection was 5 ng per 20 μl injection volume. Known amounts of tolmetin sodium were added to the pooled plasma and the exudate from the rat receiving an intraperitoneal injection of acetic acid, and analysed for tolmetin content. The recovery of tolmetin ranged from 98.5 to 99.3%. The results were not corrected for this recovery rate.

Materials

1-Methyl-*p*-toluoylpyrrole-2-acetic acid sodium dihydrate (tolmetin sodium, Figure 1), aspirin, salicylic acid and morphine hydrochloride were used. Drugs dissolved in saline (0.9% w/v NaCl solution) or suspended in a 0.5% gum tragacanth aqueous solution were administered to the rat intravenously and intraperitoneally or orally, respectively. Doses are represented in terms of each salt. One hundred mg tolmetin sodium is equivalent to 81.57 mg tolmetin (1-methyl-*p*-toluoylpyrrole-2-acetic acid).

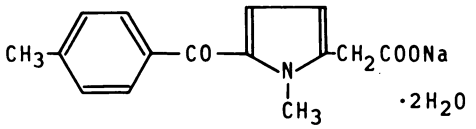

Figure 1 Structural formula of tolmetin sodium

Statistics

When writhings were 50% or less that of the vehicle control group, the dose was considered to be effective. The anti-writhing ED_{50} was calculated from the effective rates (% of number of rats in which dose effective/number of rats tested) according to the method of Litchfield & Wilcoxon (1949). The potency rate was calculated according to the method of Finney (1952) and statistical significance was determined by Student's *t* test. All values are given as mean ± s.e.mean.

Results

Time-course of anti-writhing activity of tolmetin sodium

Anti-writhing ED_{50} values of tolmetin sodium and aspirin have been reported to be 3.01 and 25.0 mg/kg orally, respectively, in the rat acetic acid-writhing test (Nakamura *et al.*, 1979b). Tolmetin sodium 3 mg/kg or aspirin 25 mg/kg was given orally to the rat 15, 30, 45 and 55 min after acetic acid injection and 45, 30, 15 and 5 min later, respectively, anti-writhing activity was measured.

As shown in Figure 2a, tolmetin sodium produced potent anti-writhing activity 5 min after dosing and the inhibitory rates (81–90.8%) when given between 30 and 55 min after acetic acid injection were significantly ($P < 0.01$) larger than that (57.1%) when given 15 min after injection. Thus, tolmetin sodium produced an immediate action. As seen in Figure 2b, aspirin had a tendency to be most active 5 min after dosing.

Tolmetin levels in plasma and exudate after oral administration

When the exudate volume was measured 60 min after injecting the rat with 1 ml of saline or a 1% acetic acid aqueous solution (i.p.), the mean exudate volume ± s.e.mean in the acetic acid-treated rats was 1.54 ± 0.17 ml ($n = 5$) and 0.33 ± 0.03 ml ($n = 4$) in the saline-treated rats. Little exudate was obtained from the normal rats. Thus, it was found that an exudative inflammation was brought about in the peritoneal cavity of the rat after acetic acid injection.

As shown in Figure 3, tolmetin content of the

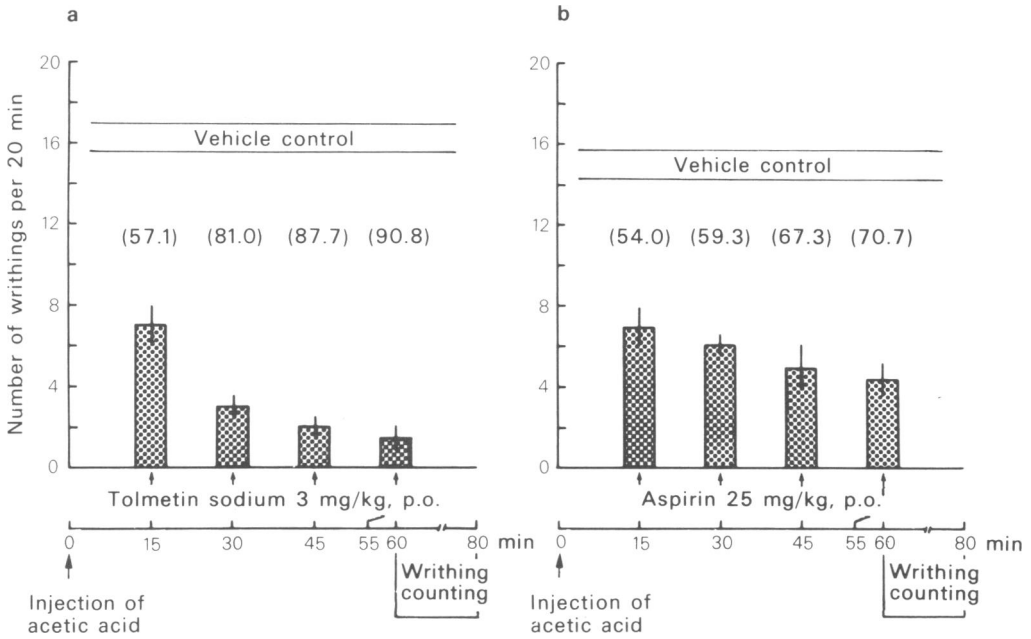

Figure 2 Time course of anti-writhing activity of tolmetin sodium (a) and aspirin (b) in rats. Tolmetin sodium 3 mg/kg or aspirin 25 mg/kg was administered orally to the rat 15, 30, 45 and 55 min after intraperitoneal injection of 1 ml of a 1% acetic acid aqueous solution and 60 min later, the number of writhings was counted for 20 min. Each column and vertical bar represent the mean and s.e.mean from 10 rats. Number of writhings of vehicle control groups was 16.3 ± 0.7 ($n = 15$) and 15.0 ± 0.7 ($n = 10$) for tolmetin sodium and aspirin, respectively. Percentage inhibition compared with vehicle control group is shown in parentheses.

exudate was 2.96 ± 0.73 μg ($n = 6$) 25 min after oral administration of 5 mg/kg of tolmetin sodium in the acetic acid-treated rats, while it was 0.61 ± 0.12 μg ($n = 5$) in normal rats. Subsequently, tolmetin levels in plasma and in the peritoneal exudate were determined at various times after oral administration of tolmetin sodium in the acetic acid-treated rats, as follows. Five mg/kg of tolmetin sodium was given orally to the rat 15, 30, 45 or 50 min after intraperitoneal injection of acetic acid, and 45, 30, 15 or 10 min later, respectively, tolmetin levels in plasma and the exudate were determined. As shown in Figure 4, tolmetin levels (7.09 ± 0.71 μg/ml in plasma; 4.64 ± 0.44 μg/rat in the exudate) at 30 min after dosing were larger than those (2.77 ± 0.70 μg/ml in plasma; 2.02 ± 0.39 μg/rat in the exudate) at 45 min after dosing. The time course in plasma coincided roughly with the tolmetin content in the exudate. However, the difference in tolmetin contents between plasma and exudate at 45 min after dosing was relatively small, in comparison with that at 10 and 15 min after dosing. This finding may be considered to be due to late movement of tolmetin from the exudate into the plasma. Thus there was a relatively large quantity of tolmetin in the peritoneal cavity of the rat receiving tolmetin sodium orally and the exudate tolmetin content corresponded approximately to its anti-writhing activity.

Comparison of anti-writhing activity of tolmetin sodium by intraperitoneal and intravenous administration

Tolmetin sodium was injected into the peritoneal cavity or into the tail vein 60 min after acetic acid injection and the number of writhings was counted for 20 min. The writhing counts of the control groups treated with saline intraperitoneally and intravenously were 13.2 ± 1.0 ($n = 13$) and 15.5 ± 1.7 ($n = 15$), respectively. Tolmetin sodium produced a dose-dependent decrease in the writhing counts; the inhibitory rates were 20.5%, 44.7% ($P < 0.01$ significantly different from the control) and 78.0% ($P < 0.01$) at intraperitoneal doses of 0.5, 1 and 5 μg/kg, respectively, and were 33.6%, 58.7% ($P < 0.01$) and 79.4% ($P < 0.01$) at intravenous doses of 50, 100 and 200 μg/kg, respectively ($n = 10$ each). The effective rates, percentages of number of

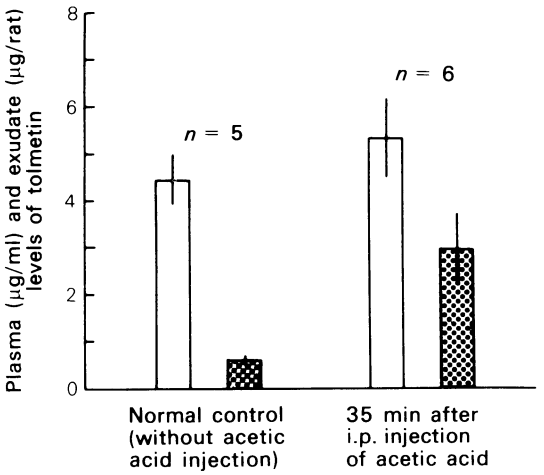

Figure 3 Comparison between tolmetin contents in the peritoneal exudate and plasma of the rat with and without intraperitoneal injection of acetic acid. Tolmetin sodium 5 mg/kg was administered orally to normal rats and to rats that had received an intraperitoneal injection of 1 ml of a 1% acetic acid aqueous solution 35 min beforehand; 25 min later, tolmetin contents in the exudate were determined. Each column and vertical bar represent the mean and s.e.mean from 5 to 6 rats; open columns = concentration in plasma, stippled columns = concentration in exudate.

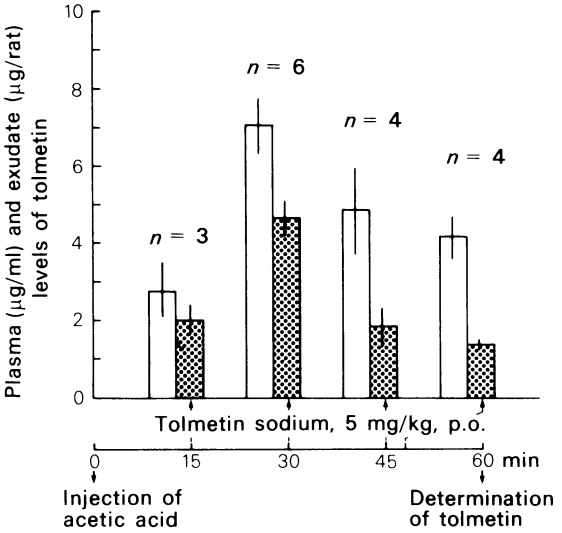

Figure 4 Tolmetin contents of plasma and peritoneal exudate of rats given tolmetin sodium, 5 mg/kg orally at 15, 30, 45 and 50 min after intraperitoneal injection of 1 ml of a 1% acetic acid aqueous solution; both the tolmetin contents were determined 60 min after acetic acid injection. Each column and vertical bar represent the mean and s.e.mean from 3 to 6 rats. Open columns = plasma levels of tolmetin; stippled columns = exudate levels of tolmetin.

rats in which dose effective/number tested, are shown in Figure 5. Anti-writhing ED_{50} (95% confidence limits), calculated from the effective rates was 1.42 (0.82–2.91) μg/kg intraperitoneally and 92.0 (57.0–140) μg/kg intravenously, and the potency ratio (95% confidence limits) of tolmetin sodium by intraperitoneal as compared to intravenous administration was 40.0 (18.5–80.2), $n = 60$. Subsequently, the anti-writhing activity of tolmetin sodium after inraperitoneal administration was 40 times more potent than that after intravenous administration. Anti-writhing ED_{50} of salicylic acid was 400 (206–775) μg/kg intraperitoneally, $n = 30$ and 7725 (3924–15207) μg/kg intravenously, $n = 25$, and its potency ratio by intraperitoneal as compared to intravenous administration was 19.4, $n = 60$. In contrast, morphine hydrochloride showed the same anti-writhing activity by both intraperitoneal and intravenous routes of administration, and its anti-writhing ED_{50} was 12.5 (7.60–20.3) μg/kg, $n = 18$.

Tolmetin levels in plasma and exudate after intraperitoneal and intravenous administration

Equipotent doses (5 μg/kg i.p.; 200 μg/kg i.v.) of tolmetin sodium were given intraperitoneally or intravenously to the rat 60 min after acetic acid injection and 3 and 20 min later, tolmetin levels in plasma and the peritoneal exudate were determined. Tolmetin contents in the exudate were 350 ± 36 ng/rat ($n = 5$) and 375 ± 31 ng/rat ($n = 5$) at 3 min after intraperitoneal and intravenous administration, respectively, and were 268 ± 12 ng/rat and 645 ± 35 ng/rat at 20 min after administration, respectively. On the other hand, the plasma level at 20 min after intravenous administration was 684 ± 37 ng/ml and was approx. 15 ng/ml after intraperitoneal administration (Figure 6). Thus, the tolmetin level in the exudate during the 20 min period after intraperitoneal administration was 0.4 to 1 times that after intravenous administration. In contrast, the difference in the plasma levels was more than 40 times. These results suggest strongly that the site of anti-writhing action of tolmetin sodium is mainly in the peritoneal cavity.

Discussion

Analgesics are generally considered to act centrally, peripherally or both centrally and peripherally. Narcotic analgesics such as morphine produce their analgesic actions by a central mechanism (Jaffe & Martin, 1975). On the other hand, it has been re-

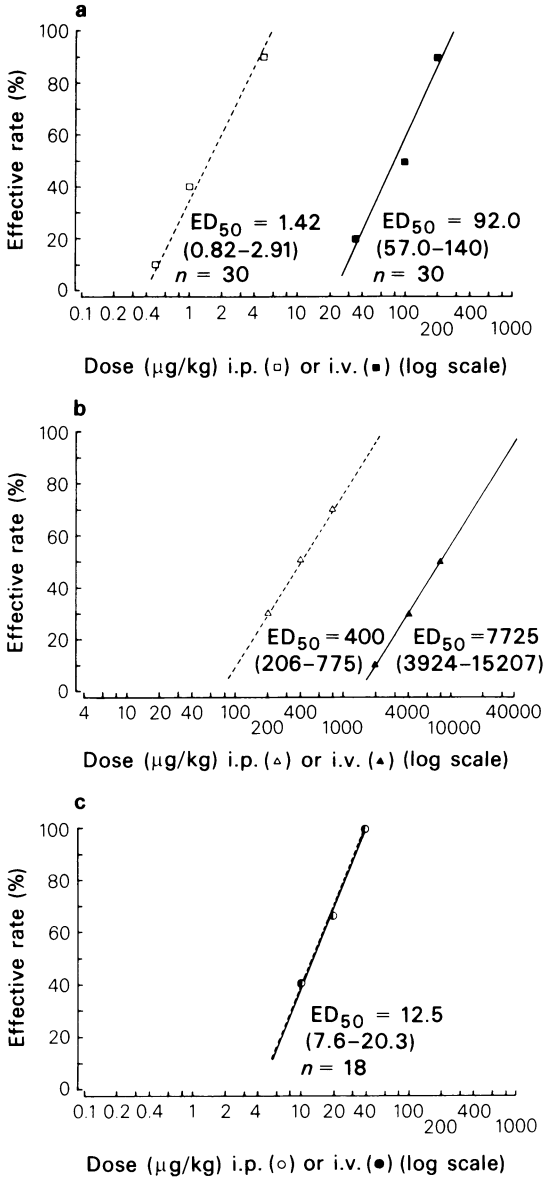

Figure 5 Comparison of anti-writhing activities of tolmetin sodium (a), salicylic acid (b) and morphine (c) by intraperitoneal and intravenous administrations in rats. Each drug was administered intraperitoneally (□; broken line) or intravenously (■; complete line) to the rat 60 min after acetic acid injection, and number of writhings was counted for 20 min. When the rat showed half or less the number of writhings counted in the vehicle control group, the dose was considered to be effective. Ordinate scale represents the percentages of number of rats in which dose effective/number of rats tested.

ported that anti-pyretic analgesics, such as aspirin, act peripherally (Guzman, Braun, Lim, Potter & Rodgers, 1964; Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Engle, 1964; Hashimoto, Kumakura & Taira, 1964; Hirata, Tanaka, Taira & Hashimoto, 1966; Lim, 1968; Taira, Nakayama & Hashimoto, 1968). However, it is supposed that salicylates produce their analgesic actions partly by a central mechanism (Winder, 1959; Dubas & Parker, 1971) and some influences of aminopyrine on the electroencephalograph have also been reported (Ban, 1963; Sasa, Fukuda & Takaori, 1969). The site of the analgesic action of most non-steroidal anti-inflammatory drugs is not clear, although some of them are considered to block impulse generation at the chemoreceptors in the periphery. The site of analgesic action of aspirin has been investigated principally by methods involving the intra-arterial injection of bradykinin in dogs. These studies are roughly divided into two groups: (1) the pain-producing site is crossperfused by the blood of the donor dog and test drugs are given intravenously to the donor and recipient dogs, and (2) test drugs are administered into the vein and bradykinin into the artery of the same dog. In both the methods, the difference in analgesic potency by different routes of administration is estimated. These are excellent methods but are not very convenient. We used a simpler analgesic assay method, the rat acetic acid-writhing test in this investigation.

It is considered that tolmetin sodium produces its analgesic action mainly by a peripheral mechanism since this drug, like aspirin, has anti-inflammatory and anti-pyretic activities (Wong *et al.*, 1973; Shimizu *et al.*, 1975a; Nakamura *et al.*, 1979b) but has no effect on the central nervous system (Shimizu, Matsuno, Soji, Kawashima, Ito & Hori, 1975b). If tolmetin sodium acts peripherally, a sufficient quantity of tolmetin to produce anti-writhing action should be found in the peritoneal cavity after oral administration and also, the anti-writhing action of tolmetin sodium should be produced by intraperitoneal (local) administration of lower doses than those used for intravenous (systemic) administration. On the other hand, the anti-writhing action of morphine after intravenous administration is considered to be equipotent to or more potent than that after intraperitoneal administration, when the same dose of morphine is given.

Tolmetin was found in the peritoneal exudate after oral administration of tolmetin sodium and the time course of the change in tolmetin concentration corresponded roughly to that of its anti-writhing activity when the period of counting (20 min) for writhing was taken into consideration (Figures 2 and 3). The potency of tolmetin sodium after intraperitoneal administration was 40 times that after intravenous ad-

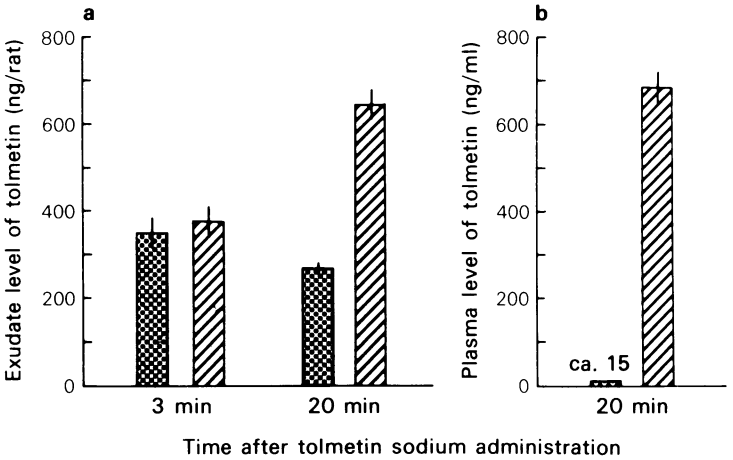

Figure 6 Exudate (a) and plasma (b) tolmetin levels afer intraperitoneal or intravenous administration of equipotent doses of tolmetin sodium in rats. Equipotent doses (5 μg/kg i.p. stippled columns; 200 μg/kg i.v. hatched columns) of tolmetin sodium were administered intraperitoneally or intravenously to the rat 60 min after acetic acid injection, and 3 and 20 min later tolmetin contents in the peritoneal exudate (a) or plasma (b) were determined. Each column and vertical bar represent the mean and s.e.mean from 5 rats.

ministration. Subsequently, tolmetin sodium produced anti-writhing activity at doses of up to 10 μg/kg (i.p.) at which dose there was no activity after intravenous administration. These results suggest that the site of anti-writhing action of tolmetin sodium is in the peritoneal cavity. However, the possibility that tolmetin sodium also acts centrally, cannot be excluded by these results.

When equipotent doses (5 μg/kg i.p.; 200 μg/kg i.v.) of tolmetin sodium were administered intraperitoneally and intravenously, the tolmetin content of both exudates was almost equal over the counting period of 20 min (Figure 6). In contrast, the difference in tolmetin concentration in the plasma after administration by these routes was more than 40 times. It has been reported that the blood level of tometin (13.3 μg equivalent/ml), is the second highest after the kidney level and is about 34 times that in the brain at 30 min after a single oral dose of [^{14}C]-tolmetin (10 mg/kg) (Hashimoto, Miyazaki, Fujii, Nambu & Tanaka, 1979). This finding suggests that the tolmetin concentration in the brain after intraperitoneal administration is much lower than that in the intraperitoneal exudate. In this experiment, if test drugs have local anaesthetic activity, the drugs are considered to produce a potent anti-writhing activity after intraperitoneal administration. However, it has been reported that tolmetin sodium has no local anaesthetic activity (Shimizu *et al.*, 1975b). These results indicate that it is very unlikely that tolmetin sodium produces its anti-writhing action by a central mechanism.

It is concluded that tolmetin sodium produces its anti-writhing action mainly by a peripheral mechanism, and that the technique of estimating the potency difference between intraperitoneal and intravenous administration of the test drug is useful for investigation of the site of action of analgesic drugs.

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