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Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism

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1 The antinociceptive effects of systemically-administered procaine, lignocaine and bupivacaine were examined in mice and rats by using the hot-plate, writhing and tail flick tests.

2 In both species all three local anaesthetics produced significant antinociception which was prevented by atropine (5 mg kg^{-1}, i.p.) and by hemicholinium-3 (1 μg per mouse, i.c.v.), but not by naloxone (3 mg kg^{-1}, i.p.), α-methyl-*p*-tyrosine (100 mg kg^{-1}, s.c.), reserpine (2 mg kg^{-1}, i.p.) or atropine methylbromide (5.5 mg kg^{-1}, i.p.).

3 Atropine (5 mg kg^{-1}, i.p.) which totally antagonized oxotremorine (40 μg kg^{-1}, s.c.) antinociception did not modify morphine (5 mg kg^{-1}, s.c.) or baclofen (4 mg kg^{-1}, s.c.) antinociception. On the other hand, hemicholinium, which antagonized local anaesthetic antinociception, did not prevent oxotremorine, morphine or baclofen antinociception.

4 Intracerebroventricular injection in mice of procaine (200 μg), lignocaine (150 μg) and bupivacaine (25 μg), doses which were largely ineffective by parenteral routes, induced an antinociception whose intensity equalled that obtainable subcutaneously. Moreover, the i.c.v. injection of antinociceptive doses did not impair performance on the rota-rod test.

5 Concentrations below 10^{-10} M of procaine, lignocaine and bupivacaine did not evoke any response on the isolated longitudinal muscle strip of guinea-pig ileum, or modify acetylcholine (ACh)-induced contractions. On the other hand, they always increased electrically-evoked twitches.

6 The same concentrations of local anaesthetics which induced antinociception did not inhibit acetylcholinesterase (AChE) *in vitro*.

7 On the basis of the above findings and the existing literature, a facilitation of cholinergic transmission by the local anaesthetics is postulated; this could be due to blockade of presynaptic muscarinic receptors.

Introduction

It has been reported that various local anaesthetics (procaine, lignocaine, bupivacaine), administered by a slow intravenous route, as well as orally-administered tocainide, suppress certain types of pain in humans. These include neoplastic pain (Cavallini & Beltrami, 1968), post-operative pain (McLachlin, 1945; Keats *et al.*, 1951; De Clive-Löwe, 1958; Bartlett & Hutaserani, 1961; Nalda Felipe *et al.*, 1977; De Gaudio *et al.*, 1978; Marchisio, 1980), post-traumatic pain (Schnapp, 1981), neuralgic pain (Boas *et al.*, 1982; Lindblom & Lindström, 1984), muscular pain (Usubiaga *et al.*, 1967; Haldia *et al.*, 1973), adiposa dolorosa (Iwane *et al.*, 1976; Lindström & Lindblom, 1987; Kastrup *et al.*, 1987; Petersen & Kastrup, 1987), labour pains (Gilbert *et al.*, 1951) and pain due to administration of radiological contrast media (Gerlock *et al.*, 1979).

While the analgesic action of local anaesthetics administered by systemically peripheral routes in humans is well-documented, even if it is not reported in the main pharmacological textbooks, very little basic research has been carried out on animals (Moore & Burney, 1979; Wiesenfeld-Hallin & Lindblom, 1985).

The precise mechanism of the analgesic action of local anaesthetics given systemically is unknown. Woolf & Wiesenfeld-Hallin (1985) reported that lig-

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nocaine and tocainide are able to suppress selectively the C-fibre-evoked polysynaptic reflex as do narcotic opiates, while Moore & Burney (1979) found that the analgesic effect of lignocaine was not antagonized by naloxone.

Methods

Hot-plate test

Male albino Swiss and NMRI mice (25–30 g) from the Morini breeding farm were used. The mice were placed inside a stainless steel container thermostatically set at 52.5 ± 0.1°C in a precision water-bath from KW Mechanical Workshops, Siena, Italy. The reaction time (s) was measured with a stop-watch before, and 15, 30 and 45 min after, treatment. The endpoint used was the licking of the fore or hind paws. Those mice which scored below 12 and over 18 s in the pre-treatment test were rejected. An arbitrary cut-off time of 45 s was adopted.

Writhing test

Male albino mice (25–30 g) were injected i.p. with a 0.5% aqueous solution of acetic acid (10 ml kg^{-1}). The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Tail-flick test

Male albino Wistar rats (250–300 g), purchased form Morini, were used. An analgesimeter from the Galileo Workshops, Florence, Italy, was used to perform the tail-flick test described by D'Amour & Smith (1941), with minor modifications.

The light from a projection bulb situated beneath the platform where the animal was placed, was focused through a small hole on the ventral part of the tail at a point about 4 cm from the tip. Withdrawal of the tail exposed a photocell to the light, which turned off thermal stimulus and automatically stopped the clock, measuring the latency period to the nearest 0.1 s. The intensity was regulated so that the reaction time varied between 3 and 5 s. A cut-off time of 10 s was used to prevent blistering.

The analgesia was tested before, and 15, 30 and 45 min after, treatment with local anaesthetics. Each value was derived from the mean of three consecutive readings in which the light was focused on three adjacent points of the tail.

Rota-rod test

Male albino Swiss mice (25–30 g) were tested on a rota-rod treadmill (Ugo Basile, Varese, Italy). The apparatus consisted of a base platform and a rotating rod of 3 cm in diameter with a non-slippery surface. This rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 discs. Thus, up to 5 mice were tested simultaneously on the apparatus, using a rod-rotating speed of 16 r.p.m. The integrity of motor coordination and of resistance to fatigue were assessed on the basis of the endurance time of the animals on the rotating rod.

Three days before the test, the animals were trained 2–3 times a day. At the end of the three days, only the mice able to stay balanced on the rotating rod for at least 120 s (cut-off time) were selected. On the fourth day, the performance time was measured before, and 15, 30 and 45 min after, treatment.

Isolated longitudinal muscle strip of guinea-pig ileum

The myenteric plexus longitudinal muscle was prepared according to Paton & Vizi (1969). The strip was suspended in a 12.5 ml thermoregulated (36–37°C) bath and stimulated by an electrical field at 0.1 Hz, 1 ms pulse duration and supramaximal voltage.

The Krebs-Henseleit solution, bubbled with 95% O_2 and 5% CO_2, had the following composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.5, KH_2PO_3 1.2, $NaHCO_3$ 25.0 and glucose 11.0.

Acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was assayed according to Ellman *et al.* (1961), using 0.5 mM acetylthiocholine iodide as substrate. The local anaesthetic inhibitory effect was tested at various concentrations on a purified preparation of AChE from the electric eel.

Drugs and reagents

The following drugs were used: procaine hydrochloride, atropine sulphate, atropine methylbromide, hemicholinium-3 and DL-α-methyl-*p*-tyrosine methyl ester hydrochloride (Sigma), reserpine and L-(+)-ascorbic acid (Merck), (+)-amphetamine sulphate (Recordati), oxotremorine (Fluka A.G.), bupivacaine hydrochloride (Pierrel), lignocaine hydrochloride (USL 10/D, Florence), naloxone hydrochloride (Endo Lab.), morphine hydrochloride (Carlo Erba), baclofen (Ciba-Geigy), 5,5′-dithiobis-(2-nitrobenzoic acid), acetylthiocholine iodide and AChE from *Electrophorous electricus* (Boehringer). Other chemicals were of the highest quality commercially available. All the drugs were dissolved in physiological saline solution, except procaine, which was dissolved in distilled water, and reserpine, which was dissolved in a 20% solution

of ascorbic acid. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml kg^{-1} by both s.c. and i.p. injection. The doses given in the text are expressed as salts.

I.c.v. administrations were performed, during short ether anaesthesia, by injecting the necessary dose dissolved in 5 μl or a maximum of 7.5 μl per mouse.

Statistical analysis

Results are given as the mean ± s.e.mean. Dunnett's two-tailed test was used to verify the significance of differences among the means, and these were considered significant when P values were less than 0.05.

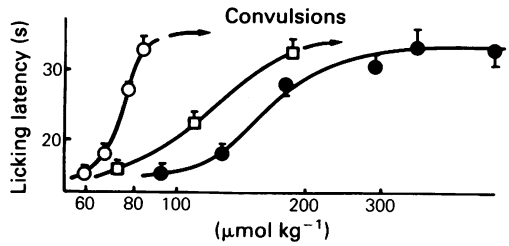

Figure 1 The antinociceptive effect of procaine (●), lignocaine (□) and bupivacaine (○) tested in mice on hot plate (52.5°C) 15 min after s.c. treatment. Each point represents the mean of at least 10 animals. Vertical lines give s.e.mean.

Results

Antinociceptive effect by peripheral route

Subcutaneously-administered procaine (50 mg kg^{-1}), lignocaine (30 mg kg^{-1}) and bupivacaine (25 mg kg^{-1}) induced a significant increase in the pain threshold in both mice and rats in all three tests (Tables 1 and 2). Analgesia reached a maximum after 15 min, persisting unchanged up to 30 min and then diminishing 45 min after administration (Tables 1 and 2). An equal degree of analgesia was always obtained in whichever area of the body (hip, central back, neck) the subcutaneous injection was made.

Figure 1 shows the dose-effect curves of the three local anaesthetics tested in the mouse on the hot plate. Bupivacaine proved to be the most active analgesic of the three local anaesthetics, followed, in order, by lignocaine and procaine.

The interval of the analgesic doses was seen to be very narrow for bupivacaine, and considerably wider for procaine. Moreover, while bupivacaine and lignocaine induced convulsions at doses of 30 and 100 mg kg^{-1} s.c., respectively, procaine had no such action (Figure 1). At the optimal analgesic doses (procaine 50 mg kg^{-1} s.c., lignocaine 30 mg kg^{-1}, s.c. and bupivacaine 25 mg kg^{-1} s.c.), the normal behaviour of both the mice and rats appeared on observation to be wholly comparable to that of the controls, to the extent that the researchers, who were unaware of the treatment received by the animals, were unable to distinguish between the various groups.

However, if the mice were subjected to the rota-rod test (Table 3), the animals treated with lignocaine and bupivacaine stayed on the rota-rod for a significantly shorter time than controls. This did not occur with procaine, which did not significantly lower rota-rod performance (Table 3).

Antinociceptive effect by intracerebroventricular route

Figure 2 shows the results obtained in the mouse following the i.c.v. administration of procaine, lignocaine and bupivacaine in the hot-plate test. The analgesia obtained with the three local anaesthetics administered i.c.v. was of comparable intensity to that obtained s.c.

Antagonism of antinociception by atropine

Tables 1 and 2 show that atropine (5 mg kg^{-1} i.p.), administered 15 min before the three local anaesthetics, completely antagonized the analgesia induced in both rats and mice. This analgesia was not antagonized either by naloxone (3 mg kg^{-1} i.p.) or by atropine methylbromide (5.5 mg kg^{-1} i.p.) (Table 1). Moreover, the pretreatment of mice with a dose of α-methyl-*p*-tyrosine methyl ester (100 mg kg^{-1} i.p.) or reserpine (2 mg kg^{-1} i.p.), which was capable of antagonizing amphetamine (1 mg kg^{-1} s.c.) analgesia, did not modify local anaesthetic analgesia (Figure 3).

Experiments on amphetamine-induced antinociception were performed only with writhing tests (Figure 3), since hypermotility induced by amphetamine makes the results of hot-plate and tail-flick tests unreliable.

Figure 4 shows that doses of 5 mg kg^{-1} i.p. of atropine were needed to antagonize completely the antinociception induced by the muscarinic agonist, oxotremorine. It is also interesting to note that this dose of atropine did not interfere in any way with morphine- or baclofen-evoked analgesia.

Antagonism of analgesia by hemicholinium-3

Table 4 shows that the i.c.v. injection of hemicholinium-3 (HC-3) (1 μg per mouse), administered 5 h before analgesic drugs, totally antagonized the analgesia induced by procaine and lignocaine, and par-

Table 1 Antinociceptive effect of procaine, lignocaine and bupivacaine and atropine antagonism in hot-plate test

Pretreatment (i.p.) (15 min before treatment)	*Treatment (s.c.)*	*Hot-plate test* Licking latency in mice (s) *Before pretreatment*	*After treatment 15 min*	*30 min*	*45 min*
None	Saline 10 ml kg^{-1}	14.5 ± 0.2 (150)	15.4 ± 0.3 (150)	15.7 ± 0.3 (150)	14.6 ± 0.2 (150)
	Procaine 50 mg kg^{-1}	15.6 ± 0.3 (60)	26.9 ± 1.3** (60)	25.2 ± 1.8** (60)	17.0 ± 0.7 (60)
	Lignocaine 30 mg kg^{-1}	14.7 ± 0.4 (45)	23.8 ± 1.1** (45)	27.3 ± 1.5** (45)	20.0 ± 1.3* (45)
	Bupivacaine 25 mg kg^{-1}	14.4 ± 0.2 (83)	25.9 ± 0.9** (83)	22.8 ± 0.6** (83)	17.1 ± 0.4 (83)
Naloxone 3 mg kg^{-1} (8.2 mmol kg^{-1})	Saline 10 ml kg^{-1}	16.1 ± 0.6 (17)	17.9 ± 1.6 (17)	14.7 ± 0.9 (17)	15.4 ± 1.3 (17)
	Procaine 50 mg kg^{-1}	16.4 ± 0.4 (10)	25.4 ± 1.6** (10)	22.9 ± 1.1** (10)	18.4 ± 1.2 (10)
	Lignocaine 30 mg kg^{-1}	16.2 ± 1.0 (7)	23.7 ± 1.5** (7)	22.3 ± 1.4** (7)	17.0 ± 1.3 (7)
	Bupivacaine 25 mg kg^{-1}	14.3 ± 0.9 (9)	23.8 ± 1.7** (9)	23.8 ± 1.8** (9)	17.0 ± 1.4 (9)
Atropine 5 mg kg^{-1} (15 mmol kg^{-1})	Saline 10 ml kg^{-1}	14.8 ± 0.3 (65)	13.9 ± 0.9 (65)	14.1 ± 0.5 (65)	14.8 ± 0.8 (65)
	Procaine 50 mg kg^{-1}	15.3 ± 0.6 (33)	16.4 ± 1.6 (33)	16.1 ± 1.9 (33)	15.7 ± 1.5 (33)
	Lignocaine 30 mg kg^{-1}	16.0 ± 0.4 (20)	17.0 ± 1.3 (20)	16.3 ± 1.4 (20)	15.2 ± 1.0 (20)
	Bupivacaine 25 mg kg^{-1}	15.4 ± 0.5 (36)	13.1 ± 1.4 (36)	15.2 ± 1.8 (36)	15.3 ± 1.7 (36)
Atropine methylbromide 5.5 mg kg^{-1} 15 mmol kg^{-1}	Saline 10 ml kg^{-1}	16.1 ± 0.5 (10)	15.8 ± 2.1 (10)	14.8 ± 2.0 (10)	16.2 ± 1.6 (10)
	Procaine 50 mg kg^{-1}	15.5 ± 0.9 (10)	24.4 ± 1.3** (10)	25.0 ± 1.6** (10)	19.1 ± 1.1 (10)
	Lignocaine 30 mg kg^{-1}	15.9 ± 1.1 (10)	25.5 ± 1.6** (10)	23.7 ± 1.1** (10)	18.1 ± 1.9 (10)
	Bupivacaine 25 mg kg^{-1} s.c.	14.4 ± 0.8 (10)	23.9 ± 1.1** (10)	19.7 ± 1.9** (10)	15.6 ± 1.0 (10)

The number of mice is shown in parentheses.
*$P < 0.05$; **$P < 0.01$; in comparison with saline controls.

tially antagonized bupivacaine-induced analgesia.

On the other hand, the analgesia induced by oxotremorine, morphine and baclofen was not modifed by i.c.v. HC-3 pretreatment.

Effect on longitudinal muscle strip of guinea-pig ileum

The effect of procaine, lignocaine and bupivacaine proved to be biphasic. At lower concentrations (below 10^{-10} M), the three local anaesthetics increased the electrically-induced twitches of the longitudinal muscle strip of guinea-pig ileum by 12.2 ± 2.0%, 37.1 ± 14.1% and 14.2 ± 3.7% respectively for procaine, lignocaine and bupivacaine (Figure 5) and did not affect the contractions produced by exogenous ACh. Moreover, the same doses did not exert any effect on non-stimulated ileum. At higher concentrations (above 10^{-5} M), however, the anaesthetics drastically reduced the responses to both electrical stimulation (Figure 5) and exogenous ACh.

Interaction with acetylcholinesterase

Procaine, lignocaine and bupivacaine partially inhibited AChE (57, 28 and 18% respectively) only at concentrations (10^{-3} M) that were considerably higher

Table 2 Antinociceptive effect of procaine, lignocaine and bupivacaine and atropine antagonism in writhing and tail-flick tests

Pretreatment (i.p.)	*Treatment (s.c.)*	*Writhing in mice (writhes 10 min^{-1}* 15 min after treatment	*Tail flick in rats (s)* Before pretreatment	15 min	After treatment 30 min	45 min
None	Saline 10 ml kg^{-1}	53.4 ± 1.9 (48)	4.4 ± 0.2 (15)	4.4 ± 0.2 (15)	4.2 ± 0.2 (15)	4.5 ± 0.1 (15)
	Procaine 50 mg kg^{-1}	31.5 ± 2.8** (14)	3.7 ± 0.1 (4)	5.5 ± 0.2** (4)	5.1 ± 0.3 (4)	4.1 ± 0.2 (4)
	Lignocaine 30 mg kg^{-1}	32.8 ± 3.7** (12)	4.0 ± 0.2 (5)	6.5 ± 0.2** (5)	6.0 ± 0.3* (5)	5.2 ± 0.1 (5)
	Bupivacaine 25 mg kg^{-1}	30.4 ± 3.5** (17)	3.5 ± 0.1 (5)	6.1 ± 0.3** (5)	4.6 ± 0.1 (5)	4.8 ± 0.8 (5)
Atropine 5 mg kg^{-1} (15 mmol kg^{-1})	Saline 10 ml kg^{-1}	50.2 ± 3.5 (17)	3.9 ± 0.3 (5)	4.0 ± 0.3 (5)	3.6 ± 0.1 (5)	3.3 ± 0.2 (5)
	Procaine 50 mg kg^{-1}	—	4.2 ± 0.1 (5)	4.0 ± 0.2 (5)	3.5 ± 0.1 (5)	3.4 ± 0.3 (5)
	Lignocaine 30 mg kg^{-1}	47.6 ± 3.4 (8)	—	—	—	—
	Bupivacaine 25 mg kg^{-1}	49.5 ± 2.1 (10)	3.9 ± 0.1 (5)	4.3 ± 0.2 (5)	3.3 ± 0.1 (5)	3.4 ± 0.2 (5)

The number of mice is shown in parentheses.
*$P < 0.05$; **$P < 0.001$; in comparison with saline controls.

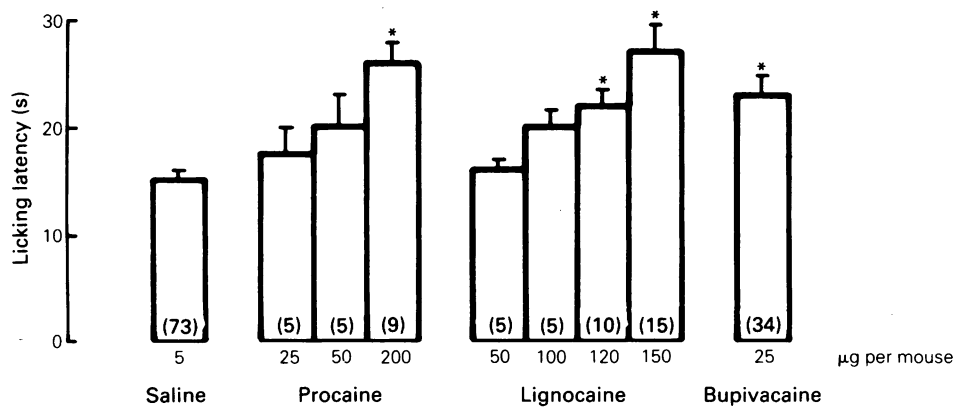

Figure 2 Antinociceptive effect of i.c.v. injection of procaine, lignocaine and bupivacaine in mice tested on hot plate (52.5°C). Test was performed 15 min after i.c.v. injection. Each column represents mean of paw-licking latency with vertical lines showing s.e.mean. The number of animals is shown in parentheses.
*$P < 0.01$ significantly different from controls.

than those necessary both for the analgesic effect *in vivo* and for the potentiation of electrically-evoked contraction *in vitro*.

Discussion

The present results indicate that procaine, lignocaine and bupivacaine, when systemically administered, produce antinociception in laboratory animals agreeing with analgesia reported in man.

The local anaesthetic-induced antinociceptive effect was always obtained, whichever test was used. Moreover, analgesia was produced in animals without altering their normal performance in the rota-rod test, and lignocaine and bupivacaine which, unlike procaine, impaired the rota-rod test, did not exert this side-effect when the administration was i.c.v. The

Table 3 Rota-rod performance of mice after subcutaneous and intracerebroventricular administration of procaine, lignocaine and bupivacaine

Treatment s.c.	*No of mice*	*Endurance time on rota-rod (s)* *Before treatment*	*After treatment* 15 min	30 min	45 min
Saline 10 ml kg^{-1}	12	98.4 ± 6.0	102.0 ± 7.5	98.4 ± 6.8	110.0 ± 4.5
Procaine 50 mg kg^{-1}	8	116.7 ± 2.8	85.4 ± 9.9	96.5 ± 8.2	107.5 ± 4.8
Lignocaine 30 mg kg^{-1}	9	114.4 ± 3.9	41.1 ± 10.9**	66.4 ± 17.1*	94.0 ± 9.6
Bupivacaine 25 mg kg^{-1}	17	117.2 ± 1.3	30.6 ± 7.7**	63.3 ± 7.7**	90.8 ± 7.9
i.c.v.					
Saline 5–7.5 µl per mouse	20	119.7 ± 0.3	106.2 ± 4.7	106.8 ± 5.6	113.2 ± 3.4
Lignocaine 150 µg per mouse	10	120.0 ± 0	110.4 ± 3.0	111.0 ± 3.5	113.2 ± 3.6
Bupivacaine 25 µg per mouse	10	120.0 ± 0	95.7 ± 9.8	110.7 ± 6.9	114.1 ± 3.1

*$P < 0.05$; **$P < 0.01$; in comparison with saline controls.

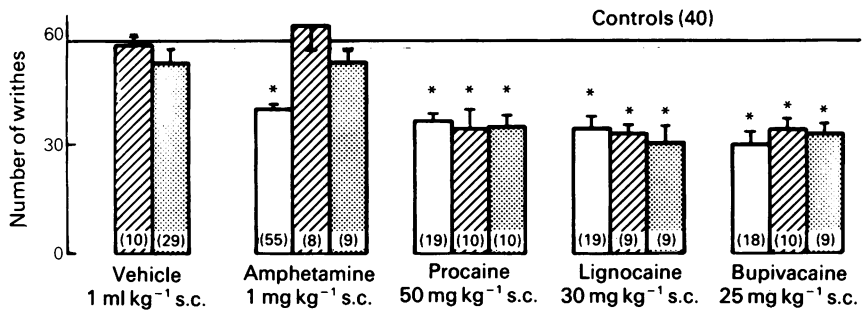

Figure 3 The effect of α-methyl-*p*-tyrosine methyl ester (hatched columns) (100 mg kg^{-1}, i.p.) and reserpine (stippled columns) (2 mg kg^{-1}, i.p.) on amphetamine (1 mg kg^{-1}, s.c.), procaine (50 mg kg^{-1}, s.c.), lignocaine (30 mg kg^{-1}, s.c.), bupivacaine (25 mg kg^{-1}, s.c.) antinociception in the mouse writhing test. α-Methyl-*p*-tyrosine methyl ester was injected 2 h before, and reserpine 48 h before, the other drugs. Nociceptive responses were recorded 10 min after administration of amphetamine and 15 min after procaine, lignocaine and bupivacaine. Vehicle represents either saline or 20% ascorbic acid solution. The histogram referring to the vehicle, therefore, represents the means of the two control groups of mice pretreated respectively with α-methyl-*p*-tyrosine methyl ester and reserpine. Vertical lines show s.e.mean. *$P < 0.01$ in comparison with saline controls. Number of mice in parentheses.

following reasoning leads to the suggestion that the site of local anaesthetic analgesia is the CNS: (1) It was possible to reach the same intensity of analgesia by injecting a dose of local anaesthetic which was considerably lower than that needed parenterally, directly into the cerebral ventricles. Dependence of the analgesia on a retrodiffusion of the drug from the cerebral ventricles to the periphery can thus be ruled out. Moreover, i.c.v. administration of hemicholinium was able to antagonize that analgesia. (2) Unlike atropine,

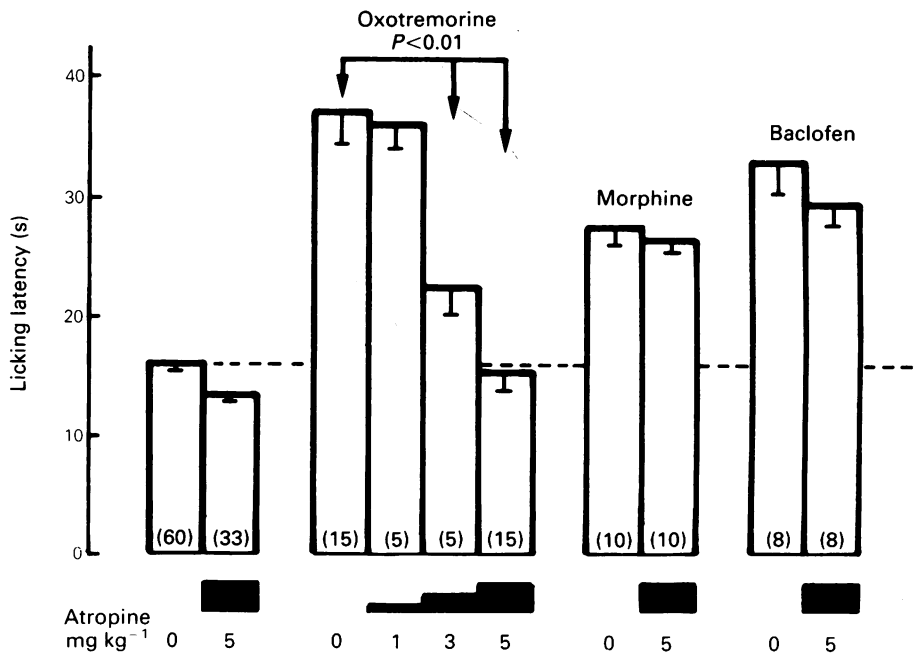

Figure 4 Effect of atropine on oxotremorine (40 μg kg^{-1}, s.c.), morphine (5 mg kg,$^{-1}$ s.c.) and baclofen (4 mg kg^{-1}, s.c.) antinociception in mouse hot-plate test (52.5°C). Atropine was injected i.p. 5 min before the other drugs. Nociceptive responses were recorded 30 min after oxotremorine and morphine, and 60 min after baclofen administration. Number of mice in parentheses. Vertical lines give s.e.mean.

Table 4 Antagonistic effect of i.c.v. hemicholinium-3 on procaine, lignocaine, and bupivacaine analgesia, in contrast to the lack of effect on oxotremorine, morphine and baclofen antinociception, in mice during hot-plate test

Pretreatment (i.c.v 5 h before treatment)	*Treatment (s.c.)*	*Licking latency in mice (s)*			
		Before treatment	*After treatment* 15 min	30 min	45 min
Saline (5 µl per mouse)	Saline 10 ml kg^{-1}	16.8 ± 0.9 (26)	16.0 ± 0.5 (26)	15.7 ± 0.5 (26)	16.4 ± 0.8 (26)
	Procaine 50 mg kg^{-1}	15.3 ± 1.1 (10)	23.2 ± 0.9** (10)	20.3 ± 0.9** (10)	17.7 ± 0.8* (10)
	Lignocaine 30 mg kg^{-1}	16.0 ± 1.7 (10)	23.1 ± 1.0** (10)	25.6 ± 1.4** (10)	20.2 ± 1.02** (10)
	Bupivacaine 25 mg kg^{-1}	15.3 ± 1.5 (10)	24.3 ± 1.7** (10)	22.4 ± 1.7* (10)	17.4 ± 1.1 (10)
	Oxotremorine 40 µg kg^{-1}	14.9 ± 0.6 (10)	25.5 ± 1.4** (10)	24.8 ± 2.2** (10)	20.6 ± 1.3* (10)
	Morphine 5 mg kg^{-1}	15.6 ± 1.1 (10)	22.1 ± 1.4** (10)	27.8 ± 1.2** (10)	27.1 ± 1.1** (10)
	Baclofen 4 mg kg^{-1}	15.9 ± 1.2 (10)	20.1 ± 1.3* (10)	25.0 ± 0.8** (10)	25.5 ± 1.0** (10)
Hemicholinium (1 µg per mouse)	Saline 10 ml kg	16.2 ± 1.8 (26)	15.4 ± 0.8 (26)	16.2 ± 0.6 (26)	15.3 ± 0.7 (26)
	Procaine 50 mg kg^{-1}	14.4 ± 1.9 (10)	17.9 ± 1.5 (10)	15.5 ± 1.6 (10)	16.4 ± 1.0 (10)
	Lignocaine 30 mg kg^{-1}	14.8 ± 1.6 (10)	17.7 ± 1.0 (10)	18.9 ± 2.3 (10)	17.2 ± 1.3 (10)
	Bupivacaine 25 mg kg^{-1}	15.7 ± 1.5 (10)	19.9 ± 1.9* (10)	17.0 ± 1.6 (10)	16.2 ± 0.8 (10)
	Oxotremorine 40 µg kg^{-1}	15.3 ± 0.6 (10)	26.9 ± 0.6** (10)	23.3 ± 1.7** (10)	26.3 ± 1.0** (10)
	Morphine 5 mg kg^{-1}	16.1 ± 1.1 (10)	19.7 ± 1.2* (10)	24.6 ± 1.7** (10)	23.9 ± 1.2** (10)
	Baclofen 4 mg kg^{-1}	16.0 ± 1.3 (10)	21.8 ± 1.4* (10)	24.3 ± 1.3** (10)	26.3 ± 1.2 (10)

The number of animals is shown in parentheses.
*$P < 0.05$; **$P < 0.01$; in comparison with saline controls.

its quaternary derivative (atropine methylbromide), which is incapable of crossing the blood-brain barrier, was not able at an equimolar dose to antagonize the analgesia induced by systemically-administered local anaesthetics.

These findings allow a change in the interpretation of the mechanism of the analgesic action of local anaesthetics in man. Analgesia evoked by systemic administration of local anaesthetics has so far been considered to depend on a local anaesthesia of the endoreceptors (stabilization of nerve-ending nociceptors) (Zipf & Dittmann, 1971), and thus to be of peripheral origin.

These results show that local anaesthetic analgesia also depends on central activation of the cholinergic system, and in fact it was possible to block it either by inhibiting muscarinic receptors with atropine or by antagonizing choline uptake with HC-3.

On the other hand, drugs with no anticholinergic activity such as naloxone, α-methyl-*p*-tyrosine and reserpine were not able to modify this analgesia by interfering with opioid, catecholaminergic and 5-hydroxytryptaminergic systems.

Although it is well-known that both direct and indirect muscarinic agonists such as oxotremorine (George *et al.*, 1962; Bartolini *et al.*, 1980) and its precursor: tremorine (Lenke, 1968), arecoline (Herz, 1962) and physostigmine (Harris *et al.*, 1959) induce antinociception in the laboratory animal; a huge difference exists between the analgesia induced in animals with local anaesthetics and that induced by the above-mentioned muscarinic agonists. While local anaesthetics produce analgesia without any visible side-effects, the muscarinic agonists provoke at the same time a clear cholinergic symptomatology (tremors, sialorrhoea, diarrhoea, rhinorrhoea,

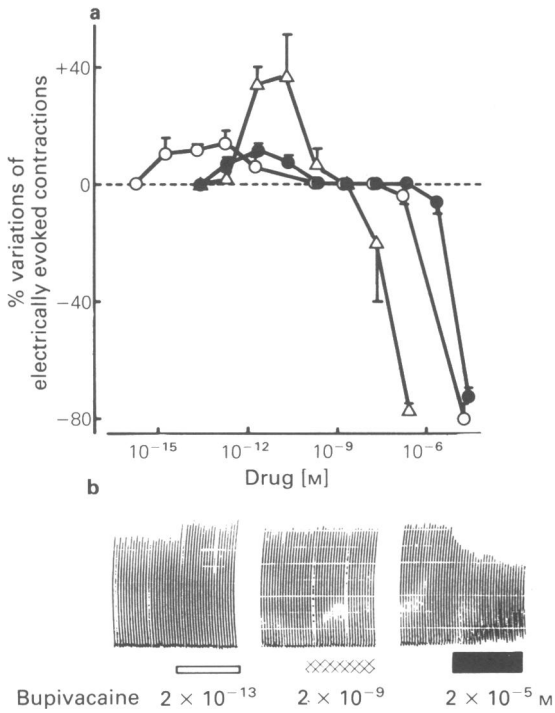

Figure 5 Effect of graduated doses of local anaesthetics on electrically-evoked contractions of guinea-pig ileum myenteric plexus longitudinal muscle strip (field stimulation: 0.1 Hz, 1 ms duration, supramaximal voltage). (a) Dose-response curves of procaine (●), lignocaine (△) and bupivacaine (○). Each point represents the mean of 4 experiments and vertical lines give s.e.mean. (b) Tracing from typical experiment.

lacrimation, etc.). Moreover, local anaesthetics did not contract isolated guinea-pig ileum, and it may thus be supposed that though the muscarinic system is involved in the analgesic effect of the local anaesthetics, the precise mechanism of action of the latter does differ from that of direct muscarinic agonists.

A presynaptic mechanism facilitating cholinergic transmission might therefore be involved in local anaesthetic analgesia. The data concerning HC-3-antagonism and the potentiation of guinea-pig-ileum-evoked contractions support this hypothesis. Our results, in fact, show that HC-3 was able to antagonize local anaesthetic but not oxotremorine-induced analgesia. This rules out the possibility of local anaesthetics acting on postsynaptic muscarinic receptors, and suggests an indirect cholinomimetic action. Since an AChE-inhibition can also be ruled out, because only doses greatly exceeding the analgesic ones produced inhibition, and isolated guinea-pig ileum contractions induced by exogenous ACh were not potentiated, it is likely that the indirect cholinomimetic action of local anaesthetics may depend on blockade of presynaptic acetylcholine autoreceptors.

The data regarding the effect of local anaesthetics on the electrically-evoked contractions of guinea-pig ileum are in agreement with this hypothesis: in fact, suitable doses of local anaesthetics increased the electrically-evoked contractions of longitudinal muscle strip, without modifying its basal tone.

The inhibition of L-quinuclidinyl [phenyl 4(n) − [^{3}H] benzilate] ([^{3}H]-QNB) binding (Aronstam *et al.*, 1980; Cohen-Armon *et al.*, 1985) to muscarinic ACh receptors by local anaesthetics such as procaine, lignocaine, dibucaine, dimethisoquin, piperocaine, prilocaine and tetracaine, also supports the hypothesis.

In this regard it is interesting to note that atropine (Mitchell, 1963; Szerb, 1964) and scopolamine (Bartolini & Pepeu, 1967) increase ACh release from cerebral cortex, and that this effect was attributable, according to Polak (1971), to presynaptic inhibition. Although our results would point to the hypothesis concerning local anaesthetic antagonism of the presynaptic autoreceptors as the most likely one, other possible mechanisms of action cannot be ignored.

The demonstration that procaine, lignocaine and bupivacaine induce analgesia through a central mechanism which is antagonized by atropine, and HC-3 throws new light on the controversial question of the systemic use of procaine in the treatment of the elderly. The beneficial effects of procaine on orientation, attention, memory, psychic depression, libido, appetite, euphoria and various types of pain, which were initially described by Aslan (1956) and subsequently confirmed by various authors (Ostfeld *et al.*, 1977), could be explained by the improvement of the functioning of the cholinergic system.

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