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Antinociceptive and anti-inflammatory activity of a novel quipazine derivative (AAL-13): a selective inhibitor of 5-hydroxytryptamine reuptake

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Abstract—The anti-inflammatory and antinociceptive activities of a novel quipazine derivative 2(4-(3-chloropropyl)piperazinyl) quinoline (AAL-13), a selective inhibitor of 5-hydroxytryptamine (5-HT) reuptake, has been examined. Anti-inflammatory activity was assessed by measuring the inhibition of a cotton pellet granuloma and of carrageenan-induced paw oedema in rats, and of cantharidin-induced topical inflammation in the mouse ear. Antinociceptive activity was studied by using the modified Randall-Selitto method. Indomethacin was used as a reference. AAL-13 slightly inhibited granuloma formation (13%, $P<0.02$) at 100 mg kg^{-1} day^{-1} for 7 days, whereas half that dose had no significant effect. There was significant inhibition of carrageenan-induced rat paw oedema (35%, $P<0.05$ and 103%, $P<0.001$) 3 h after single doses of AAL-13 (50 and 100 mg kg^{-1} p.o., respectively). Three hours after i.p. injection, the oedema inhibition was 58% ($P<0.05$) and 86% ($P<0.001$) for doses of 25 and 50 mg kg^{-1}, respectively. In comparison, indomethacin (3, 6 and 12 mg kg^{-1} p.o.) inhibited oedema by 59% ($P<0.02$), 65% ($P<0.01$) and 63% ($P<0.02$), respectively. Intraperitoneally, only the 12 mg kg^{-1} dose produced significant inhibition (82%, 3 h after carrageenan injection, $P<0.05$). AAL-13 (1·5 mg/ear) had a significant anti-inflammatory effect on the mouse ear (52%, inhibition, $P<0.05$), while indomethacin (3 mg/ear) gave 43% inhibition ($P<0.05$). AAL-13 raised the pain threshold and analgesic index in both inflamed and non-inflamed rat paw similarly, while indomethacin (2–3 mg kg^{-1}) was more active in the inflamed paw. However, the larger dose of indomethacin (12 mg kg^{-1}) did raise the threshold in non-inflamed limbs. AAL-13 (up to 100 mg kg^{-1} daily for 7 days) was devoid of any ulcerogenic effect on the stomach, while indomethacin was lethal in doses greater than 3 mg kg^{-1} daily. We postulated that the anti-inflammatory and antinociceptive activity of AAL-13 might be due to its ability to block 5-HT and noradrenaline reuptake.

A novel quipazine derivative 2(4-3(chloropropyl)piperazinyl)-quinoline (AAL-13) synthesized in our laboratory (Alhaider et al 1985) has been found to be a selective inhibitor of 5-hydroxytryptamine (5-HT) reuptake. Its IC50 for 5-HT reuptake inhibition was 0·53 μM ± 0·03 s.e.m. compared with 5 μM for noradrenaline (Alhaider 1987). We have here examined the potential antinociceptive and anti-inflammatory activities of this compound.

Materials and methods

Male Wistar rats, 150–200 g, and male mice, 30–40 g, were used. The AAL-13 was given in solution in water either orally or intraperitoneally. As a reference, indomethacin was given in suspension in 1% methyl cellulose orally, or in solution in ethanol (10 mg mL^{-1}) intraperitoneally. The vehicle for topical application of both drugs was a mixture of ethanol, acetone and ether (1:2:3 v/v).

Inhibition of granuloma formation was measured according to Meier et al (1955). Two 50 mg cotton pellets were implanted subcutaneously, one on each side of the rat abdomen. They were removed together with granuloma 7 days later and weighed. Drugs were given orally in 1 mL of vehicle.

Inhibition of carrageenan-induced oedema in rat hind paws was measured using the method of Winter et al (1962). All rats were hydrated orally with 5 mL of water, then carrageenan (0·05 mL, 0·1%) was injected in the right hind paws 1 h following drug administration either orally (9 groups of animals) or intraperitoneally (8 groups). Foot volume was measured using a plethysmometer (Apelex-France) before and 3 h after carrageenan injection in the orally-treated rats and 3, 5 and 7 h after carrageenan injection for intraperitoneally-treated animals.

Topical anti-inflammatory activity was assessed by use of cantharidin-induced inflammation in the mouse ear with a method similar to that of Boris & Hurley (1977). Groups of mice (5–8) were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.). Cantharidin 0·5 mg/0·1 mL of vehicle (ethanol–acetone–diethyl-ether 1:2:3 v/v) was applied alone or with drugs on both the outer and the inner surfaces of one ear. After 72 h, a punch was taken from each ear and the net increase in weight and the percentage inhibition of inflammation estimated.

The antinociceptive activity of the drugs was determined in rats by the modified Randall-Selitto method (Winter & Flataker 1965). One hind paw was injected with 0·1 mL of 1% suspension of carrageenan. Two hours later, drugs were given orally in 1 mL of the vehicle and the pain threshold measured (1, 3 and 4 h) using an Analgesy-Meter (Apelex-France).

Statistics. The significance of the results was tested by using analysis of variance and Student's *t*-test.

Results

Inhibition of granuloma formation. The results of assessing the anti-inflammatory activity of AAL-13 in inhibiting granuloma formation in rats are presented in Table 1, and are compared with the effects of indomethacin. The 50 mg kg^{-1} dose of AAL-13 was ineffective, and doubling the dose inhibited granuloma formation by only 13%. Indomethacin was much more potent than AAL-13 in this experiment ($P<0.01$).

Inhibition of carrageenan-induced oedema. Oral administration of AAL-13 (50 and 100 mg kg^{-1}) inhibited oedema 35 and 103%,

Table 1. Effect of the quipazine derivative (AAL-13) and indomethacin on cotton pellet granuloma in rats.

Drug	Daily dose (mg kg^{-1})	n =	Granuloma dry wt (mg) Mean ± s.e.m.	Inhibition %
Controls				
Water		18	78·25 ± 2·53	—
Methylcellulose		9	100·29 ± 4·59	
AAL-13	50	10	76·14 ± 4·83	2·7
	100	10	68·39 ± 2·1**	12·6
Indomethacin	1	8	71·47 ± 2·35****	28·74
	1·8	10	63·19 ± 2·26****	37
	3·0	8	55·85 ± 3·29****	44·31

The significance of the effects as compared with control were assessed by Student's *t*-test.
** $P<0.02$; **** $P<0.001$.

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Table 2. Effect of the quipazine derivative (AAL-13) and indomethacin on carrageenan-induced rat hind paw oedema, 3 h following oral administration of the drugs.

Drug	Dose mg kg^{-1}	n =	Oedema (mL) Mean ± s.e.m.	Inhibition %
Controls				
Water		19	0·62 ± 0·06	
Methylcellulose		16	0·74 ± 0·13	
AAL-13	12·5	8	0·7 ± 0·09	
	25	8	0·74 ± 0·08	
	50	6	0·4 ± 0·1*	35·48
	100	10	−0·03 ± 0·02****	103·5
Indomethacin	3	10	0·3 ± 0·04**	59·46
	6	10	0·26 ± 0·03***	64·86
	12	8	0·27 ± 0·05**	63·5

The significance of the effects as compared with controls were assessed by Student's *t*-test.
* $P < 0·05$; ** $P < 0·02$; *** $P < 0·01$; **** $P < 0·001$.

respectively (Table 2), while indomethacin inhibition ranged from 59 to 65%. AAL-13 was more effective when it was given intraperitoneally (i.p.) rather than orally (p.o.); i.p. doses of 25 and 50 mg kg^{-1} produced 58 and 86% inhibition of oedema, respectively, whereas p.o. the equivalent treatment produced 0% and 35% inhibition (Table 3). Although AAL-13 inhibition was greatest at 3 h after carrageenan, it was still significant at 7 h. The effect of high dose AAL-13 was close to that of high dose indomethacin at all times tested.

Inhibition of mouse ear inflammation. AAL-13 was effective at doses of 1·5 mg/ear when tested on the inflamed mouse ear where it produced 52% inhibition compared with 43% after indomethacin (3 mg/ear) (Table 4).

Antinociceptive action. At 30–60 mg kg^{-1}, AAL-13 increased pain threshold in both inflamed and non-inflamed rat paws (Table 5). Indomethacin at 1–3 mg kg^{-1} raised pain threshold in

Table 3. Effect of the quipazine derivative (AAL-13) and indomethacin on carrageenan-induced rat hind paw oedema following intraperitoneal injection of the drugs.

Drug	Dose mg kg^{-1}	n =	Oedema mL (mean ± s.e.m.) 3 h	5 h	7 h	Inhibition % 3 h	5 h	7 h
Controls		16	0·72 ± 0·06	0·82 ± 0·08	0·84 ± 0·15			
AAL-13	12·5	7	0·45 ± 0·11	0·66 ± 0·13	0·51 ± 0·11	37·5	19·51	39·23
	25	8	0·30 ± 0·05*	0·44 ± 0·08***	0·42 ± 0·11***	58·33	46·34	50·0
	50	8	0·10 ± 0·03****	0·26 ± 0·06**	0·29 ± 0·08****	86·11	68·29	65·48
Indomethacin	1·5	8	0·56 ± 0·06	0·63 ± 0·05	0·76 ± 0·04	22·22	23·17	9·52
	3·0	7	0·26 ± 0·08	0·25 ± 0·03****	0·21 ± 0·06****	63·88	69·51	75
	6·0	8	0·25 ± 0·04****	0·13 ± 0·03****	0·25 ± 0·05****	65·27	84·14	70
	12·0	8	0·13 ± 0·03*	0·20 ± 0·05****	0·28 ± 0·05****	81·94	75·6	66·66

The significance of the effects as compared with controls were assessed by Student's *t*-test.
* $P < 0·05$; ** $P < 0·02$; *** $P < 0·01$; **** $P < 0·001$.

Table 4. Topical effect of the quipazine derivative (AAL-13) and indomethacin on cantharidin-induced inflammation in mouse ear observed after 72 h.

Drug	Topical dose of drug (mg) per ear in (0·1 mL) vehicle containing (0·5 mg) cantharidin	n =	Net increase in punch wt (mg) Mean ± s.e.m.	% Inhibition of punch wt
Cantharidin		5	7·0 ± 0·9	
Indomethacin	3·0	8	3·95 ± 0·6*	43·57
AAL-13	0·75	5	5·66 ± 0·6	19·14
	1·5	5	3·34 ± 0·8*	52·28

The significance of the effects as compared with controls were assessed by Student's *t*-test.
* $P < 0·05$.

Table 5. Effect of the quipazine derivative (AAL-13) on pain threshold, using rat hind paws.

Drug	Dose mg kg^{-1}	n =	Inflamed paw Pain threshold (g) Mean ± s.e.m.	Analgesic index	Non-inflamed Paw Pain threshold (g) Mean ± s.e.m.	Analgesic index
Control		8	162·71 ± 12·82		228·75 ± 13·99	
AAL-13	20	7	187·61 ± 25·1	1·15	230·71 ± 30·52	1·01
	30	8	401·67 ± 81·34****	2·47	549·99 ± 96·63***	2·40
	60	7	677·38 ± 128·47***	4·16	786·78 ± 161·15****	3·44
Indomethacin	2	6	261·11 ± 26·62***	1·60	266·22 ± 31·54	1·14
	3	8	407·39 ± 70·17****	2·51	263·33 ± 31·81***	1·15
	6	7	414·99 ± 74·32****	2·55	779·52 ± 63·897***	3·41

The significance of the effects as compared with control were assessed by Student's *t*-test.
*** $P < 0·01$. **** $P < 0·001$.

inflamed paws only, although 6 mg kg^{-1} raised the pain threshold in both inflamed and non-inflamed paws (Table 5).

None of the stomachs of rats receiving compound AAL-13, acutely or chronically, showed macroscopic evidence of congestion or ulceration. In contrast, the maximal tolerated dose of indomethacin in chronic experiments was only 3 mg kg^{-1}. In acute experiments, up to 12 mg kg^{-1} of indomethacin could be tolerated; the stomach showed areas of congestion but no ulceration.

Discussion

The results show that AAL-13 is an anti-inflammatory and antinociceptive agent. The anti-inflammatory activity was evident both in the acute exudative phase of inflammation, in rat paw and mouse ear, and in the late proliferative phase, in rat cotton pellet granuloma. On a weight basis AAL-13 was less potent than indomethacin, except in cantharidin-induced inflammation where it was more potent. Indomethacin (1·6 mg/ear) was reported to be ineffective in topical inflammation in rats by Boris & Hurley (1977). In this study it was found effective at doses of 3 mg/ear. The efficacy of AAL-13 was higher after i.p. than p.o. administration suggesting a lesser oral bioavailability. However, indomethacin had more or less equal potency by both routes, which is in agreement with its high oral bioavailability (98%) (Benet 1987).

The activity of AAL-13 in carrageenan- and cantharidin-induced oedema might appear paradoxical in the context of its inhibition of 5-HT reuptake (Alhaider 1987), since 5-HT increases vascular permeability (De Clerk et al 1984). Alternatively, it might be postulated that suppression of exudative inflammation by AAL-13 is due to inhibition of noradrenaline reuptake (Alhaider 1987), thereby producing vasoconstriction and reduction of capillary permeability.

The antinociceptive effect of AAL-13 was evident in both inflamed and non-inflamed rat paws, while indomethacin was more effective in the inflamed paws and it raised the pain threshold in non-inflamed paws only at the highest dose. This effect of AAL-13 might be attributed to blocking the reuptake of 5-HT and noradrenaline (Alhaider 1987), since α-adrenoceptor and tryptaminergic agonists have been shown to possess antinociceptive activity (Bently et al 1977; Jaffe & Martin 1985). Furthermore, clinical studies revealed the effectiveness of imipramine and tricyclic antidepressants, which are known to block reuptake of 5-HT and noradrenaline, in the treatment of patients with chronic pain, migraine, tension headache, neuropathic pain and cancer pain (Merskey & Hester 1972; Kocher 1976; Clifford 1985; Feinmann 1985).

Interestingly, AAL-13 was devoid of ulcerogenic and haemorrhagic effects on the stomach, even in large doses. In contrast, indomethacin caused lethal gastric bleeding in rats given doses higher than 3 mg kg^{-1} daily.

In conclusion, the present work suggests that, subject to favourable toxicology, compound AAL-13 might represent a potentially useful new antinociceptive and anti-inflammatory drug devoid of the ulcerogenic or gastric haemorrhagic effects of currently available drugs.

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