ELSEVIER

PII S0024-3205(96)00230-5

Life Sciences, Vol. 58, No. 25, pp. 2297-2309, 1996

0024-3205/96 $15.00 + .00

CENTRAL CHOLINERGIC ANTINOCICEPTION INDUCED BY 5HT$_4$ AGONISTS: BIMU 1 AND BIMU 8

Carla Ghelardini, Nicoletta Galeotti, Fiorella Casamenti, Petra Malmberg-Aiello, Giancarlo Pepeu, *Fulvio Gualtieri and Alessandro Bartolini

Departments of Preclinical and Clinical Pharmacology, Viale G.B. Morgagni 65, I-50134 Florence and *Pharmaceutical Sciences, Via G. Capponi 9, I-50121 Florence, Italy

(Received in final form April 17, 1996)

Summary

The antinociceptive effect of two 5-HT$_4$ agonists, BIMU 1 and BIMU 8, were examined in mice and rats by using the hot-plate, abdominal constriction and paw-pressure tests. In both species, BIMU 1 (10-20 mg kg^{-1} s.c.and 40-60 mg kg^{-1} p.o. in mice; 20 mg kg^{-1} i.p. in rats) and BIMU 8 (20-30 mg kg^{-1} s.c. and 60 mg kg^{-1} p.o. in mice; 20 mg kg^{-1} i.p. in rats), produced significant antinociception which was prevented by atropine (5 mg kg^{-1} i.p.), hemicholinium-3 (1 µg per mouse i.c.v.), SDZ 205-557 (10 mg kg^{-1} i.p.), GR 125487 (20 mg kg^{-1} i.p.) but not by naloxone (1 mg kg^{-1} i.p.), CGP 35348 (100 mg kg^{-1} i.p.) and reserpine (2 mg kg^{-1} i.p.). Moreover, BIMU 1 and BIMU 8 increase of pain threshold, is abolished by nucleus basalis magnocellularis (NBM) lesions in rats. SDZ 205-557 and GR 125487 which totally antagonized BIMU 1 and BIMU 8 antinociception did not modify morphine (7 mg kg^{-1} s.c.) or baclofen (4 mg kg^{-1} s.c.) antinociception. Intracerebroventricular injection in mice of BIMU 1 (3 µg per mouse) and BIMU 8 (10 µg per mouse), doses which were largely ineffective by parenteral routes, induces an antinociception whose intensity equaled that obtainable s.c., i.p. or p.o. In the antinociceptive dose-range, neither 5HT$_4$ agonist impaired mice motor coordination evaluated by rota-rod test. On the basis of the above data, it can be postulated that BIMU 1 and BIMU 8 exerted an antinociceptive effect mediated by a central amplification of cholinergic transmission.

Key Words: cholinergic system, antinociception, BIMU 1, BIMU 8, 5-HT$_4$ agonist

The stimulation of adenylate cyclase by 5HT$_4$ agonists triggers a cascade of cellular events thought to lead to the release of neurotransmitters such as acetylcholine (ACh) (1). 5HT$_4$ agonists are able

Correspondence should be addressed to: Prof. Alessandro Bartolini, Department of Pharmacology, Viale G.B. Morgagni , 65, I-50134 Florence, Italy

to stimulate guinea-pig ileum motility through activation of intramural cholinergic nerve pathways subserving peristalsis (2, 3) since this effect is abolished by antimuscarinic treatment (3). BIMU 1 and BIMU 8 are two benzimidazolone derivatives which exhibit $5HT_4$ agonistic properties (4, 5). These two compounds are endowed not only with the ability to potentiate electrically stimulated twitch responses in guinea-pig ileum longitudinal (6) and circular muscle (3), but also to stimulate the contractility of the gastric pouch in conscious dogs (7, 8) and detrusor muscle in human (9). Moreover, BIMU 1 and BIMU 8 facilitate in vivo ACh release in the frontal cortex of rats as shown by microdialysis studies (10). This facilitatory effect was blocked by the selective $5HT_4$ antagonists GR 125487 and GR 113808 indicating, therefore, that this potentiation of ACh release is mediated by $5HT_4$ receptors. It has long been known that ACh (11), selective M_1 agonists such as McN-A-343 and AF-102B (12), unselective muscarinic agonists such as tremorine (13), oxotremorine (14, 15), arecoline (16), pilocarpine (17) and cholinesterase inhibitors such as physostigmine (18, 19) and diisopropyl phosphorofluoridate (20) induce antinociception in laboratory animals by the activation of the cholinergic system. Moreover, the amplification of cholinergic neurotransmission induced by antagonism of muscarinic autoreceptors (21, 22, 23) or, alternatively, by interaction with heteroreceptors (24) located on presynaptic cholinergic terminals, produces a central antinociceptive effect. In the present work, the ability of BIMU 1 and BIMU 8 for modifying pain threshold was investigated.

Methods

Animals

Male Swiss albino mice (23-30 g) and Wistar rats (200-300 g) from the Morini breeding farm were used. Fifteen mice and four rats were housed per cage. The cages were placed into the experimental room 24 h before the test for acclimatization. The animals were kept at 23±1 °C with a 12 h light/dark cicle, light at 7 a.m., with food and water ad libitum. Animals were randomly assigned to a control (saline solution) or a treated group (BIMU 1 - BIMU 8). Both groups received a pretreatment consisting in the injection of one of the following antagonists: atropine, naloxone, SDZ 205-557, GR 125487, hemicholinium-3 (HC-3), CGP-35348 or reserpine. All the antagonists were injected 15 min before treatment with the exception of HC-3, CGP 35348 and reserpine. HC-3 and CGP-35348 were administered respectively 5 h and 5 min before treatment whereas reserpine was injected twice, 48 and 24 h, before the test. All animals used were drug naive. All experiments were carried out according to the guidelines of the European Community Council.

Hot plate test

The method adopted was described by O'Callaghan and Holtzman (25). Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s), were measured with a stop-watch about 30 min before pretreatment (pretest) and 15, 30 and 45 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted at which the animals were removed from the hot-plate and given a score of 45 s.

Abdominal constriction test

The test was performed in mice according to Koster et al. (26). Mice were injected i.p. with a 0.6% 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. Both BIMU 1 and BIMU 8 were administered 10 min before acetic acid injection.

Paw pressure

The nociceptive threshold in the rat was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton et al. (27). Threshold pressure was measured about 30 min before treatment (pretest) and 15, 30 and 45 min after treatment. Rats scoring below 50 g or over 85 g during the pretest were rejected (25%). An arbitrary cut-off value of 250 g was adopted.

Rota-rod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. This rod was placed at height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. (28). The performance time was measured before and 15, 30 and 45 min after treatment.

Nucleus basalis magnocellularis lesions

Under ketamine anaesthesia (100 mg kg^{-1} i.p.) bilateral lesions of the rat nucleus basalis magnocellularis (NBM) were made by stereotaxic injection of 0.5 µl of 0.12 M quisqualic acid dissolved in 50 mM sodium phosphate buffer (pH=7.4) with a 10 µl Hamilton syringe. The injection lasted 3 min and the syringe was left in position for 5 min after the completion of the infusion. The following coordinates, taken from Paxinos and Watson (29) stereotaxic atlas, were used: 0.5 mm posterior to bregma, 2.8 mm lateral, 6.8 mm below dura. Twenty days after surgery the weight of the rats was only slightly below that of controls and they looked healthy. In the sham-operated rats the syringe needle was lowered into the cortex and no quisqualic acid was injected. At the end of the experiments the location and size of the lesions were checked by histological examination on 10 µm-thick slices stained with the cresyl violet according to the Nissl method.

Choline acetyltransferase determination

After completing the behavioural paw-pressure test, lesioned and sham operated rats were decapitated. Brains were then removed and cortexes dissected out. Choline acetyltransferase activity was determined according to the method of Fonnum (30).

Drugs

The following drugs were used: BIMU 1 (endo-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazol-1-carboxamide hydrochloride), BIMU 8 (endo-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazol-1 carboxamide hydrochloride) and GR 125487 ([1-[2(methylsuphonyl)amino]ethyl]-4-piperidinyl] methyl- 5- fluoro - 2-methoxy-1H-indole-3-carboxylate hydrochloride) (Boehringer Ingelheim, Italy), atropine sulphate, baclofen and quisqualic acid (Sigma, USA), hemicholinium-3 hydrobromide (HC-3) and naloxone hydrochloride (RBI), SDZ 205-557 (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester hydrochloride) was prepared in the Department of Pharmaceutical Sciences of University of Florence according to the method described by Romanelli et al. (31), morphine hydrochloride (Carlo Erba, Italy), CGP 35348 (3-aminopropyl-diethoxymethyl-phosphinic acid), ascorbic acid and reserpine (Ciba Geigy, Switzerland), sodium chloride and acetic acid glacial (Merck), sodium carboxymethylcellulose (Fluka, Switzerland). All drugs were dissolved in isotonic (NaCl 0.9 %) saline solution or dispersed in sodium carboxymethylcellulose 1% immediately before use except reserpine which was dissolved in a 20% solution of ascorbic acid. Drug concentrations were prepared in such a way that could be administered in a volume of 10 ml kg^{-1} by subcutaneous (s.c.), intraperitoneal (i.p.) and per os (p.o.) route. Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia using isotonic saline as solvent, according to the method described for mice by Haley and McCormick (32) and extended for rats by us. Substances were injected in the necessary dose dissolved in 5 µl for mice and 10 µl for rats. To ascertain the exact point into which the drugs were administered, some mice or rats were injected i.c.v. with 5 or 10 µl of diluted 1:10 Indian ink and their brains were examined macroscopically after sectioning.

Statistical analysis: Results are given as the mean ± s.e.m.; analysis of variance, followed by Scheffe's F procedure for post-hoc comparison, was used to verify significance between two means. *P* values of less than 0.05 were considered significant. Data were analyzed with computer program (StatView for the Macintosh, 1992).

Results

The antinociceptive effect of BIMU 1 and BIMU 8 was investigated on the hot-plate and abdominal constriction tests in mice and on the paw pressure test in rats. In the hot-plate test, BIMU 1 and BIMU 8, injected i.p. in the range of doses of 10-20 mg kg^{-1} and 20-30 mg kg^{-1} respectively, induced a significant increase in the pain threshold (Fig. 1 - panel A and B). The antinociceptive effect reached a maximum 15 min after administration and then diminished, disappearing within 45 min. Table 1 shows that BIMU 1 and BIMU 8 antinociception was completely prevented by the antimuscarinic drug atropine (5 mg kg^{-1} i.p.), the choline uptake blocker HC-3 (1 µg per mouse i.c.v.) and both 5-HT_4 antagonists SDZ 205-557 (10 mg kg^{-1} i.p.) and GR 125487 (20 mg kg^{-1} i.p.). Conversely, no modification in BIMU 1 and BIMU 8 antinociception was obtained by pretreating mice with the opioid antagonist naloxone (1 mg kg^{-1} i.p.) and the $GABA_B$ antagonist CGP 35348 (100 mg kg^{-1} i.p.). Fig. 2 shows that the doses of 10 mg kg^{-1} i.p. and 20 mg kg^{-1} i.p. of SDZ 205-557 and GR 125487 respectively were needed

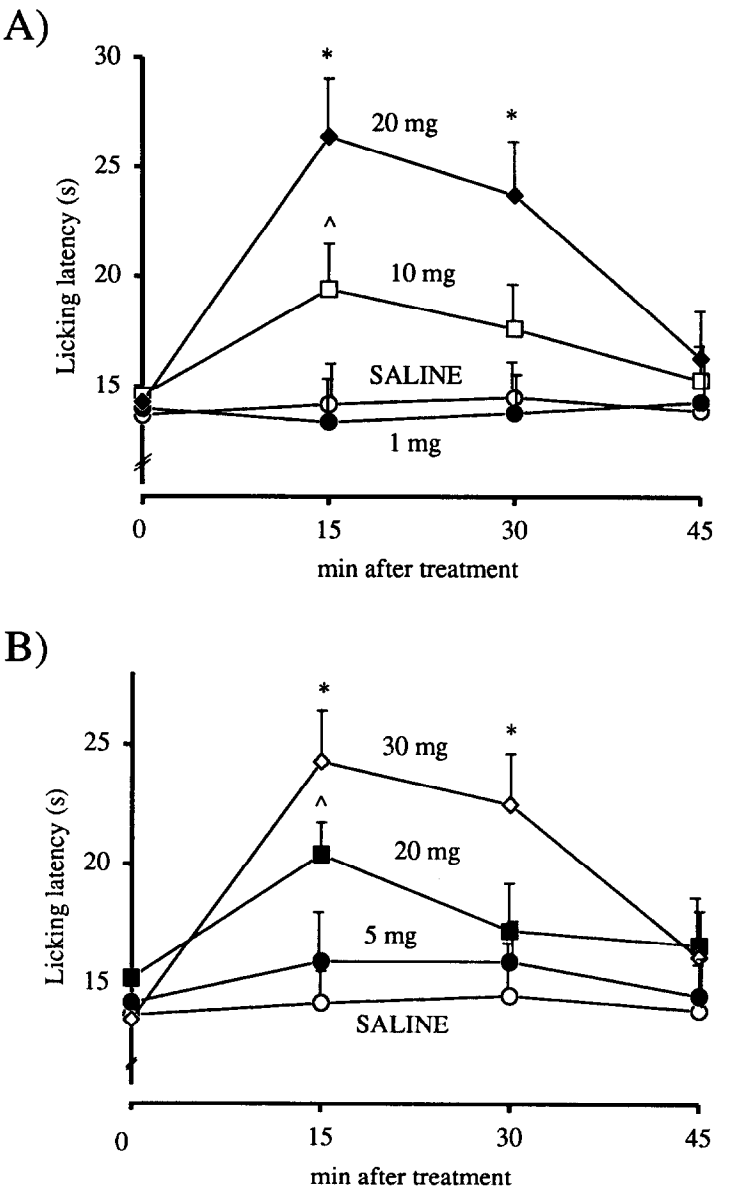

Fig. 1

Dose response curves of BIMU 1 (*panel A*) and BIMU 8 (*panel B*) in the mouse hot-plate test.
The doses are expressed as $mg \cdot kg^{-1}$ i.p.. Vertical lines show s.e. mean. ^P< 0.05; * P < 0.01 in comparison with saline controls. Each point represents the mean of at least 10 mice.

TABLE 1

Effects of atropine, HC-3, naloxone, CGP-35348, SDZ-205557 and GR 125487 on antinociception caused by BIMU 1 and BIMU 8 in the mouse hot-plate test.

			Licking latency (s)			
Pretreatment	*Treatment*	$mg \cdot kg^{-1}$ s.c.	*Before pretreatment*	*After treatment 15 min*	*30 min*	*45 min*
Saline $10\ ml \cdot kg^{-1}$ i.p.	Saline		13.6±0.7	14.4±1.0	13.9±0.9	14.3±0.8
Saline 5 µl i.c.v.	Saline		14.1±0.9	13.9±1.2	13.3±0.9	14.0±1.2
Saline i.p. or i.c.v.	BIMU 1	20	14.2±0.9	26.4±1.8*	23.7±1.6*	16.3±1.6
	BIMU 8	30	13.5±1.1	24.3±1.7*	22.5±1.5*	17.1±1.3
Atropine $5\ mg \cdot kg^{-1}$ i.p.	Saline		14.0±0.7	13.3±1.3	13.1±1.1	14.2±1.3
	BIMU 1	20	14.2±1.0	16.7±1.8°	16.1±2.2°	15.0±1.8
	BIMU 8	30	13.8±1.1	16.1±2.1°	15.9±1.9°	14.2±1.7
HC-3 1µg per mouse i.c.v.	Saline		14.4±0.9	13.8±1.5	15.5±1.3	15.4±1.2
	BIMU 1	20	13.9±1.1	17.1±2.0°	16.6±1.9°	14.2±1.5
	BIMU 8	30	14.0±1.2	16.6±1.8°	17.6±1.7°	15.4±2.2
Naloxone $1\ mg \cdot kg^{-1}$ i.p.	Saline		13.5±0.8	14.0±1.5	13.9±1.6	14.3±1.7
	BIMU 1	20	13.8±0.7	27.1±2.3*	24.7±2.3*	18.3±2.4
	BIMU 8	30	14.1±0.9	26.1±1.7*	25.1±2.1*	17.8±1.8
CGP 35348 $100\ mg \cdot kg^{-1}$ i.p.	Saline		13.5±0.7	11.4±1.3^	12.5±2.0	12.7±1.5
	BIMU 1	20	14.4±0.8	25.5±1.8*	23.8±2.2*	16.0±1.6
	BIMU 8	30	13.8±1.0	22.3±2.3*	20.6±2.3^	15.5±1.8
SDZ-205557 $10\ mg \cdot kg^{-1}$ i.p.	Saline		14.2±1.0	15.1±1.8	14.5±1.6	14.8±2.0
	BIMU 1	20	13.2±0.9	17.0±2.2°	17.1±1.9°	16.5±2.1
	BIMU 8	30	14.2±1.3	16.6±1.7°	15.6±2.2°	14.4±1.7
GR 125487 $20\ mg \cdot kg^{-1}$ i.p.	Saline		13.8±1.1	14.0±1.8	14.5±1.8	16.5±1.4
	BIMU 1	20	14.4±1.2	17.3±1.5°	17.2±2.0°	15.4±1.9
	BIMU 8	30	13.7±1.0	16.5±1.7°	16.7±2.1°	14.1±1.8

The number of mice is shown in parentheses. * $P < 0.01$; ^ $P < 0.05$ in comparison with saline-saline; ° $P < 0.01$ versus saline-BIMU 1 or saline-BIMU 8 treated mice.
The number of mice ranged from 8-26 with the exception of saline-saline were n = 28-30.

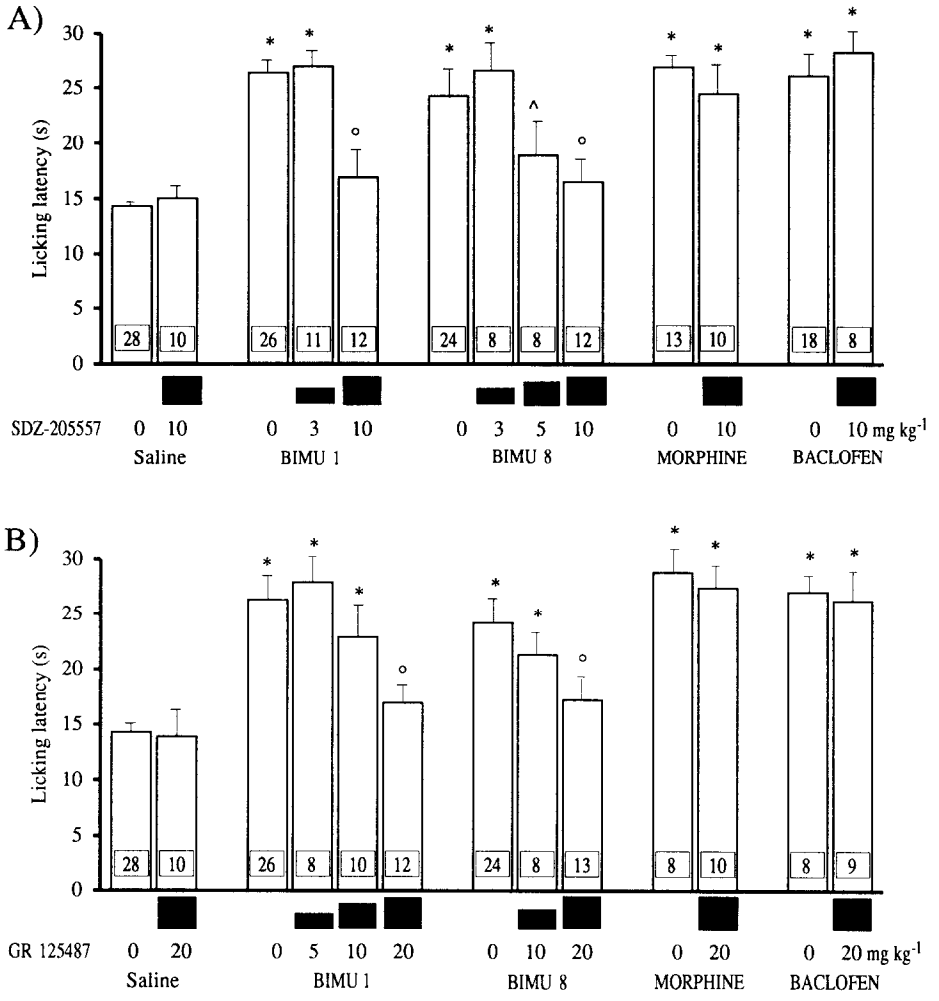

Fig. 2

Effect of SDZ-205557 (*panel A*) and GR-125487 (*panel B*) administered i.p. on antinociception induced by BIMU 1 (20 mg kg^{-1}), BIMU 8 (30 mg kg^{-1} s.c.), morphine (7 mg kg^{-1} s.c.) and baclofen (4 mg kg^{-1} s.c.) in the mouse hot-plate test. SDZ-205557 and GR-125487 were injected i.p. 15 min before BIMU 1 and BIMU 8 and 5 min before morphine and baclofen. Nociceptive responses were recorded 15 min after BIMU 1 and BIMU 8 administration and 30 min after morphine and baclofen injections. Numbers inside the columns indicate the number of mice. Vertical lines show s.e. mean. ^P< 0.05; * P< 0.01 in comparison with saline controls. ° P< 0.01 versus BIMU 1 or BIMU 8 treated mice.

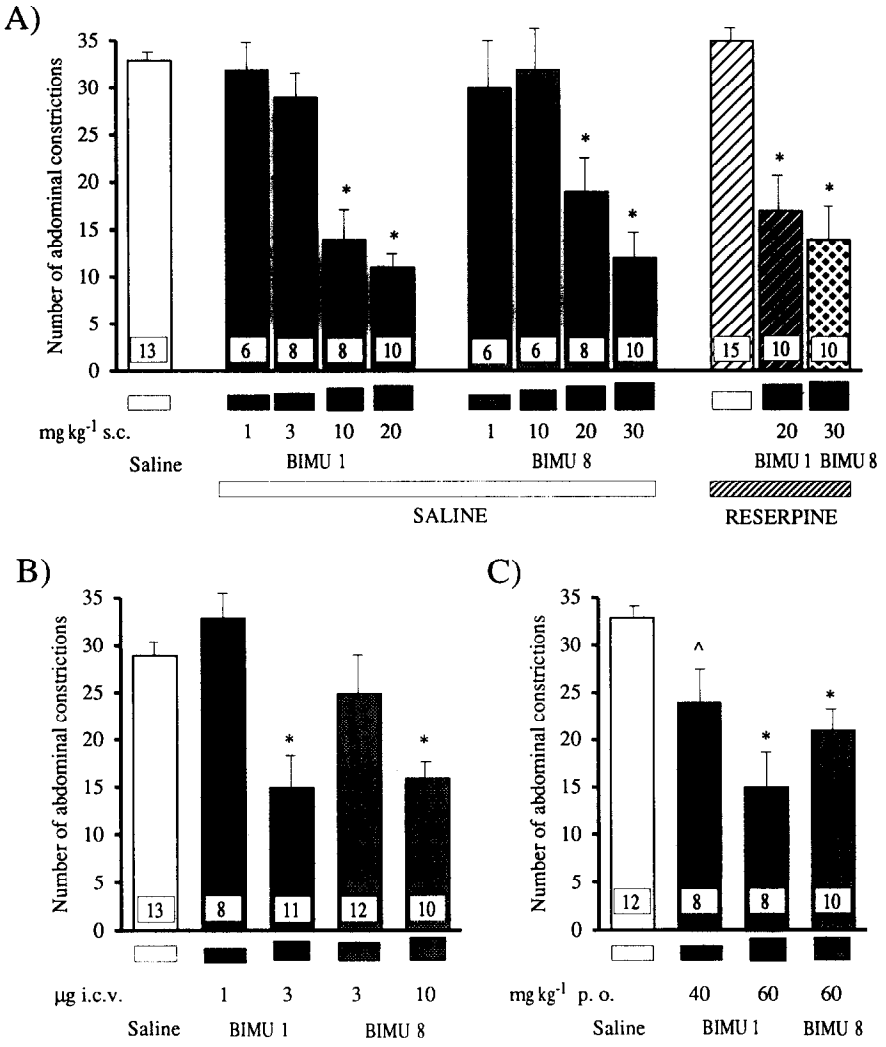

Fig. 3

Dose-response curve of BIMU 1 and BIMU 8 administered s.c. (*panel A*), i.c.v. (*panel B*) and p. o. (*panel C*) in the mouse abdominal constriction test and lack of effect by reserpine (2 mg·kg^{-1} i.p.) pretreatment on BIMU 1 and BIMU 8 induced antinociception (*panel A*).
Reserpine was injected twice 48 and 24 h before test. Vertical lines show s.e. mean. ^P< 0.05; * P< 0.01 in comparison with saline controls. The nociceptive response was recorded 15-25 min after BIMU 1 and BIMU 8 administration. Numbers inside the columns indicate the number of mice.

TABLE 2

Effect of BIMU 1 and BIMU 8 in comparison with morphine in rats with and without bilateral lesions of the nucleus basalis magnocellularis tested in the rat paw pressure test

Treatment i.p.	*PAW-PRESSURE (g)*			
	Before treatment	*After treatment* 15 min	30 min	45 min
Saline 10 ml kg^{-1}				
Naive (x)	66.6±3.4	67.5±2.4	63.4±3.2	64.4±3.4
Sham	60.4±4.0	62.8±4.2	59.4±4.2	60.6±3.6
Lesioned	64.7±4.2	65.4±3.8	60.4±4.6	58.3±3.8
BIMU 1 20 mg kg^{-1}				
Naive	69.5±3.9	112.5±5.0*	75.0±4.0	64.0±4.2
Sham	68.4±5.3	121.3±4.7*	77.4±5.2	62.3±4.8
Lesioned	71.2±3.6	79.2±4.3°	72.5±4.5	67.0±3.8
BIMU 8 20 mg kg^{-1}				
Naive	66.4±3.4	105.0±3.8*	94.6±4.8*	68.6±4.2
Sham	60.8±4.8	116.3±5.6*	102.4±4.8*	71.2±5.6
Lesioned	64.3±3.6	70.5±4.0°	62.8±4.2°	65.4±3.8
Morphine 5 mg kg^{-1}				
Naive	63.5±4.0	88.4±5.4^	133.0±5.1*	128.6±5.0*
Sham	62.4±3.4	92.3±4.5*	152.4±4.9*	139.5±5.2*
Lesioned	69.3±3.4	92.4±4.5*	138.4±6.1*	147.4±5.8*

The number of rats ranged from 6-9 with the exception of (x) where n = 13.
^P< 0.05; * P<0.01 in comparison with saline control.°P< 001 in comparison with the corresponding sham operated rats.

to completely antagonize the antinociception induced by the 5-HT$_4$ agonists BIMU 1 and BIMU 8 without interfering in any way with morphine (7 mg kg^{-1} s.c.) and baclofen (4 mg kg^{-1} s.c.) evoked analgesia. The dose-response curve of BIMU 1 and BIMU 8 administered s.c., i.c.v. and p.o. on the abdominal constriction test is shown in Fig. 3. BIMU 1 and BIMU 8 exhibit in this test an antinociceptive effect independently of the administration route. The reduction of number of abdominal constrictions caused by BIMU 1 (20 mg kg^{-1} s.c.) and BIMU 8 (30 mg kg^{-1} s.c.) was not prevented by pretreatment with reserpine (2 mg kg^{-1} i.p.), a monoamine store depletor (Fig. 3). As shown in Table 2, BIMU 1 and BIMU 8 antinociception was confirmed in

TABLE 3

Effect of BIMU 1 and BIMU 8 in the mouse rota-rod test.

Treatment	*Dose*		*Number of falls (30 s)*			
			Before pretreatment	*After treatment*		
				15 min	*30 min*	*45 min*
Saline	10	ml·kg^{-1} s.c.	3.2±0.4	1.9±0.2	1.1±0.2	0.9±0.2
BIMU 1	10	mg·kg^{-1} s.c.	3.4±0.3	1.4±0.4	1.1±0.2	0.6±0.3
BIMU 1	20	mg·kg^{-1} s.c.	3.0±0.5	1.5±0.2	1.2±0.3	0.6±0.2
BIMU 1	30	mg·kg^{-1} s.c.	3.4±0.4	2.8±0.5*	2.3±0.4*	1.3±0.3
BIMU 8	20	mg·kg^{-1} s.c.	3.1±0.4	1.3±0.3	1.1±0.3	1.1±0.2
BIMU 8	30	mg·kg^{-1} s.c.	3.5±0.5	2.3±0.3	1.6±0.4	0.8±0.3
BIMU 8	40	mg·kg^{-1} s.c.	3.6±0.4	3.6±0.3*	2.5±0.3*	1.4±0.4
Saline	5	µl i.c.v.	2.9±0.5	1.6±0.3	1.0±0.4	0.8±0.3
BIMU 1	3	µl i.c.v.	3.2±0.4	2.3±0.4	1.5±0.3	1.3±0.2
BIMU 1	10	µl i.c.v.	3.3±0.4	3.7±0.4*	3.1±0.5*	2.8±0.3*
BIMU 8	10	µl i.c.v.	3.5±0.5	1.7±0.3	1.2±0.3	1.3±0.3
BIMU 8	20	µl i.c.v.	3.5±0.4	3.4±0.4*	3.0±0.3*	2.5±0.4*
Saline	10	ml·kg^{-1} p. o.	3.4±0.5	2.8±0.3	1.8±0.4	1.6±0.3
BIMU 1	60	mg·kg^{-1} p. o.	3.2±0.5	2.5±0.4	1.8±0.3	1.0±0.3
BIMU 8	60	mg·kg^{-1} p. o	3.6±0.3	2.7±0.2	1.3±0.3	1.4±0.3

Each value represents the mean of 5 -10 mice.

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

the rat paw-pressure test where the time-course reflected that observed with the hot-plate test (Fig. 1). In the paw-pressure test, no antinociception by BIMU 1 and BIMU 8 was detected in rats with bilateral lesions of the NBM (Table 2). In these rats cortical choline acetyltranferase activity was reduced by 55 ± 6.1% (n=5) as compared with the sham operated rats. The antinociceptive effect of morphine (5 mg kg^{-1} i.p.) in the lesioned rats did not differ from that in the sham operated rats (Table 2). Finally, it should be noted that BIMU 1 and BIMU 8 elicited their antinociceptive effects without changing motor coordination as revealed by the rota-rod test where BIMU 1 and BIMU 8, administered s.c., i.c.v. and p.o. in an antinociceptive dose-range, did not increase the number of falls from the rotating rod (Table 3). Both compounds, at doses of 50 mg kg^{-1} s.c., produced convulsions and 50% of the animals died.

Discussion

BIMU 1 and BIMU 8 antinociception was elicited whichever noxious stimulus was used: thermal (hot-plate test), chemical (abdominal constriction test) and mechanical (paw pressure test). Doses which increase pain threshold were devoid of any other modification of animal behaviour. In fact, the motor coordination, evaluated by the mouse rota-rod test, was not modified by treatment with BIMU 1 and BIMU 8 at analgesic doses. 5-HT_4 agonists antinociception was found to be dependent on a cholinergic activation as this analgesia is antagonized by the muscarinic antagonist atropine, by the ACh depletor HC-3 and by lesion of the NBM which is the primary source of ACh for the cerebral cortex (33). Furthermore BIMU 1 and BIMU 8 exerted their antinociceptive effect by acting centrally since i.c.v. administration of the above-mentioned drugs were able to reduce the number of abdominal constrictions with the same intensity as that obtainable after s.c. or p.o. administration. Moreover, the antagonism exerted by i.c.v. injected HC-3 in mice and NBM lesions in rats on BIMU 1 and BIMU 8 induced antinociception, shows that the site of action of the two 5-HT_4 agonists is centrally located. The integrity of the central cholinergic system is, therefore, fundamental for BIMU 1 and BIMU 8 antinociception. To this end, it is well known that direct or indirect cholinomimetics are able to increase pain threshold in both humans (34) and animals (12, 13, 14, 15, 16, 17, 18, 19, 20). A large difference exists between the analgesia induced in animals by both BIMU 1 and BIMU 8 and that induced by direct muscarinic agonists and cholinesterase inhibitors. In fact, while 5-HT_4 agonists produce antinociception without any visible side effect, the direct muscarinic agonists and the cholinesterase inhibitors provoke, at the same time, a clear cholinergic symptomatology (tremors, sialorrhoea, diarrhoea, lacrimation etc.). Other neurotransmitter systems did not appear to be involved in BIMU 1 and BIMU 8 antinociception since the opioid antagonist naloxone, the $GABA_B$ antagonist CGP 35348 and reserpine, were all unable to prevent 5-HT_4 agonist-induced analgesic effect. The doses and administration schedules of the above-mentioned drugs were suitable for preventing antinociception induced respectively by morphine (23), $GABA_B$ agonist baclofen (35) and the antidepressant drugs clomipramine and amitriptyline (36). The prevention of BIMU 1 and BIMU 8 antinociception produced by the 5-HT_4 antagonists SDZ 205-557 (37) and GR 125487 (38) suggests, moreover, that the central serotoninergic system exerts an excitatory tone on the cholinergic system. BIMU 1 and BIMU 8, by activating serotoninergic heteroreceptors, increase ACh release as well documented by microdialysis experiments (10). The results obtained have shown a good relationship between the antinociceptive effect of BIMU 1 and BIMU 8 and the potentiation of cerebral ACh release described by Consolo et al. (10). In fact, the latency required to reach the maximum amplification of ACh release (20 min) by BIMU 1 and BIMU 8 was equal to that required for reaching their antinociceptive peak (15 min). The fact that physostigmine was used in rats employed for ACh collection from the cerebral cortex but not in those used for antinociceptive tests, could explain the more lasting effects of BIMU 1 and BIMU 8 on ACh release as compared to antinociception.

In summary our results have shown that BIMU 1 and BIMU 8 are able to induce antinociception by potentiating endogenous cholinergic activity.

Acknowledgment

This research was partially supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) and Consiglio Nazionale delle Ricerche (CNR). The authors wish to thank Boehringer Ingelheim (Milan, Italy) for their kind gift of BIMU 1, BIMU 8 and GR 125487. Preliminary data was presented at the XXIII Annual Meeting Society for Neuroscience, Washington D.C., November 7-12, 1993.

References

1. J. BOCKAERT, H. ANSANARY and C. WAEBER, CNS Drugs 1 15 (1994)
2. M. TONINI, C.A. RIZZI, L. MANZO and L. ONORI, Pharmacol.Res. 24 5-14 (1991)
3. M. TONINI, S. M. CANDURA, L. ONORI, T. COCCINI, L. MANZO and C. RIZZI, Life Sci. 50 PL173-PL178 (1992)
4. G.S. BAXTER and D.E. CLARKE, Eur. J. Pharmacol. 212 225-229 (1992)
5. A. DUMUIS, M. SEBBEN and J. BOCKAERT, Naunyn-Schmiedeberg's Arch. Pharmacol. 340 403-410 (1989)
6. C.A. RIZZI, T. COCCINI, L. ONORI, L. MANZO and M. TONINI, J. Pharmacol. Exp. Ther. 261 (2), 412-419 (1992)
7. P. SCHIANTARELLI, J. BOCKAERT, R. CESANA, A. DONETTI, A. DUMUIS, E. GIRALDO, H. LADINSKY, E. MONFERRINI, C.A. RIZZI, A. SAGRADA and A. SCHIAVONE, Pharmcol. Res. 22 Suppl. 2 453 (1990)
8. A. SCHIAVONE, R. CESANA, R. MICHELETTI, C.A. RIZZI, M. TURCONI, M. NICOLA, P. SCHIANTARELLI and A. DONETTI, Proceedings of II IUPHAR Satellite Meeting on Serotonin, Basel, Switzerland, July 11-13 p. 113 (1990)
9. M. TONINI, E. MESSORI, G.P. FRANCESCHETTI, C.A. RIZZI, A.F. CASOLDI, T. COCCINI, S.M. CANDURA, Br. J. Pharmacol. 113 1-2 (1994)
10. S. CONSOLO, S. ARNABOLDI, S. GIORGI, G. RUSSI and H. LADINSKY, NeuroReport 5 1230-1232 (1994)
11. N.W. PEDIGO, W.L. DEWEY and L.S. HARRIS, J. Pharmacol. Exp. Ther. 193, 845-852 (1975)
12. A. BARTOLINI, C. GHELARDINI, L. FANTETTI, M. MALCANGIO, P. MALMBERG-AIELLO and A. GIOTTI, Br. J. Pharmacol. 105 77-82 (1992)
13. D. LENKE, Exp. Pathol. Pharmacol. 234 35-45 (1958)
14. R. GEORGE, W.L. HASLETT, D.J. JENDEL, Life Sci. 1 361-363 (1962)
15. A. BARTOLINI, A. GALLI, C. GHELARDINI, A. GIOTTI, M. MALCANGIO, P. MALMBERG-AIELLO and P.L. ZUCCHI, Br. J. Pharmacol. 92 711-721 (1987)
16. A. HERZ, Arch. Exp. Pathol. Pharmakol. 242 414-429 (1962)
17. L.C. HENDERSHOT and J. FORSAITH, J. Pharmacol. Exp. Ther. 125 237-240 (1959)
18. L.S. HARRIS, W.L. DEWEY, J. HOWES, J.S. KENNEDY and H. PARS, J. Pharmacol. Exp. Ther. 169 17-22 (1969)
19. J.D. IRESON, Br. J. Pharmacol. 40 92-101 (1970)
20. T.L. LENTZ, L. LILEY and U. MICHAELSON, Br. J. Pharmacol. 32 156-162 (1969)
21. A. BARTOLINI, C. GHELARDINI, F. GUALTIERI, M. MALCANGIO, P. MALMBERG-AIELLO, M.N. ROMANELLI and A. GIOTTI, Trends in Pharmacol. Sci.

Suppl. IV 99 (1989)
22. F. GUALTIERI, C. GHELARDINI, A. GIOTTI, M. MALCANGIO, P.MALMBERG-AIELLO and A. BARTOLINI, Trends in Pharmacol. Sci. Suppl. IV 99 (1989)
23. C. GHELARDINI, P.MALMBERG-AIELLO, A. GIOTTI, M. MALCANGIO and A. BARTOLINI, Br. J. Pharmacol. 101 49-54 (1990)
24. C. GHELARDINI, A. GIOTTI, F. GUALTIERI, R. MATUCCI, M.N. ROMANELLI, S.SCAPECCHI, E. TEODORI and A. BARTOLINI, *Trends in Receptor Research*, P. Angeli, U. Gulini and W. Quaglia (Eds) 95-114, Eltsevier Sci. Publishers, Amsterdam (1992)
25. J.P. O'CALLAGHAN and S.G. HOLTZMAN, J. Pharmacol. Exp. Ther. 197 533-544 (1976)
26. R. KOSTER, M. ANDERSON and E.J. DE BEER, Fed. Proc. 18 412 (1959)
27. G.E. LEIGHTON, R.E. RODRIGUEZ, R.G. HILL and J. HUGHES, Br. J. Pharmacol. 93 553-560 (1988)
28. J. VAUGHT, K. PELLEY, L.G. COSTA, P. SETHER and S.J. ENNA, Neuropharmacol. 24, 211-216 (1985).
29. G. PAXINOS and G. WATSON, *The rat brain in stereo-taxic coordinates*, Academic press, New York (1982)
30. F. FONNUM, J. Neurochem. 24 407-409 (1975)
31. M.N. ROMANELLI, C. GHELARDINI, S. DEI, R. MATUCCI, F. MORI, S. SCAPECCHI, E. TEODORI, A. BARTOLINI, A. GALLI, A. GIOTTI and F. GUALTIERI, Arzneim. Forsch/Drug Res. 43 (II) n°8 913-918 (1993)
32. T.J. HALEY and G.L. McCORMICK, Br. J. Pharmacol. Chemother. 12 12-15 (1957)
33. R.T. RICHARDSON and M.R. DELONG, Trends Neurosci. 11 (6) 264-267 (1988)
34. D.D. HOOD, J.C. EISENACH and R. TUTTLE, Anestesiol. 82 331-343 (1995)
35. M. MALCANGIO, C. GHELARDINI, A. GIOTTI, P. MALMBERG-AIELLO and A. BARTOLINI, Br. J. Pharmacol. 103 1303-1308 (1991)
36. N. GALEOTTI, C. GHELARDINI and A. BARTOLINI, Behav. Pharmacol., 6 (Suppl. 1), p. 20 (1995)
37. K.H. BUCHHEIT, R. GAMSE and H.J. PFANNKUCHE, Eur. J. Pharmacol. 200 373-374 (1991)
38. C.J. GROSSMAN, G.J. KILPATRIK and K. T. BUNCE, Br. J. Pharmacol 109 618-624 (1993)