UNIVERSITÀ DEGLI STUDI FIRENZE

FLORE
Repository istituzionale dell'Università degli Studi di Firenze

THE CENTRAL CHOLINERGIC SYSTEM HAS A ROLE IN THE ANTINOCICEPTION INDUCED IN RODENTS AND GUINEA-PIGS BY THE

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

THE CENTRAL CHOLINERGIC SYSTEM HAS A ROLE IN THE ANTINOCICEPTION INDUCED IN RODENTS AND GUINEA-PIGS BY THE ANTIMIGRAINE DRUG SUMATRIPTAN / C. GHELARDINI; N. GALEOTTI; M. NICOLODI; F. SICUTERI; A. BARTOLINI; M. FIGINI; A. IMPERATO; G. GESSA. - In: THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS. - ISSN 0022-3565. - STAMPA. - 279:(1996), pp. 884-890.

Availability:

This version is available at: 2158/15985 since:

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf)

Publisher copyright claim:

(Article begins on next page)

12 October 2024

0022-3565/96/2792-0884$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 279, No. 2
Copyright © 1996 by The American Society for Pharmacology and Experimental Therapeutics
Printed in U.S.A.
JPET 279:884–890, 1996

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

The Central Cholinergic System Has a Role in the Antinociception induced in Rodents and Guinea Pigs by the Antimigraine Drug Sumatriptan[1]

CARLA GHELARDINI, NICOLETTA GALEOTTI, MICHELA FIGINI, ASSUNTA IMPERATO, MARIA NICOLODI, FEDERIGO SICUTERI, GIAN LUIGI GESSA and ALESSANDRO BARTOLINI

Department of Preclinical and Clinical Pharmacology (C.G., N.G., A.B.), University of Florence, Interuniversity Centre: Neurochemistry and Clinical Pharmacology of Idiopathic Headache (M.F., M.N., F.S.), Florence and Department of Neuroscience (A.I., G.L.G.), University of Cagliari, Italy

Accepted for publication July 18, 1996

ABSTRACT

The antinociceptive effect of the antimigraine drug sumatriptan was assessed in mice and rats (hot-plate, abdominal constriction and paw-pressure tests) and in guinea pigs (paw-pressure test). The ACh extracellular concentration also was detected in the hippocampus of freely moving rats by microdialysis experiments. Antinociception was induced by sumatriptan administered both parenterally (5–10 mg · kg^{-1} i.v.; 10–30 mg · kg^{-1} i.p.) and i.c.v. (50–100 μg per mouse). Sumatriptan antinociception was potentiated by physostigmine (0.05 mg · kg^{-1} i.p.) and was prevented by the muscarinic antagonist atropine (5 mg · kg^{-1} i.p.), the ACh depletor HC-3 (1 μg per mouse i.c.v.) and the 5-hydroxytryptamine$_{1A}$ antagonist 1-(2-methoxyphenyl)-4-[4-(2 phthalimido)butyl] piperazine (0.5 mg · kg^{-1} i.p.). Naloxone, 3-aminopropyl-diethoxy-methyl-phosphinc acid, 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester and reserpine, administered in doses suitable for blocking analgesia induced by morphine, baclofen, 5-hydroxytryptamine$_4$ agonists and clomipramine, respectively, did not modify sumatriptan antinociception. Sumatriptan, administered in the range of antinociceptive doses, was able to increase the level of ACh present in extracellular hippocampal space. On the basis of these findings, we can deduce that sumatriptan was able to induce antinociception by increasing cholinergic activation in the CNS. Such activation, as indicated by the antagonism exerted by 1-(2-methoxyphenyl)-4-[4-(2 pethalimido)butyl]piperazine, may depend on stimulation of 5-hydroxytryptamine$_{1A}$ autoreceptors. It remains to be clarified whether the antimigraine activity of sumatriptan in humans is totally dependent on cranial vessel vasoconstriction or whether its central cholinergic antinociception also plays a role.

Modulation of antinociception can occur *via* different neuronal systems. Many neuromediators, such as enkephalines, γ-aminobutyric acid, catecholamines, 5-HT and histamine, are able to induce an enhancement of the pain threshold. In particular, muscarinic antinociception can be obtainable through the stimulation of postsynaptic M_1 receptors (Bartolini *et al.*, 1992), the antagonism of muscarinic autoreceptors (M_2–M_4) and receptors (D_2, A_1 and H_3) (Ghelardini *et al.*, 1992) or the activation of 5-HT$_4$ heteroreceptors (Ghelardini *et al.*, 1993).

Recently it was reported that 8-OH-DPAT, a 5-HT$_1$ agonist endowed with high selectivity compared with 5-HT$_{1A}$ subtypes, is able to induce antinociception in rodents (Ghelardini *et al.*, 1992; 1994; Millan 1994). This naloxone-resistant antinociception is mediated by the central cholinergic system because it is prevented by ACh depletion or by atropine administration (Ghelardini *et al.*, 1994). This observation is in agreement with the results of Bianchi *et al.* (1990), which showed an increase in ACh efflux from the cerebral cortex of freely moving guinea pigs after administration of 8-OH-DPAT.

Sumatriptan (GR43175) is a novel 5-HT$_{1D}$ receptor agonist that is effective in migraine attack (Doenicke *et al.*, 1988; Humphrey and Feniuk, 1991). However, sumatriptan also has affinity for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (McCarthy and Peroutka 1989; Dechant and Clissold, 1992). In order to ascertain whether sumatriptan, in addition to cranial vessel vasoconstriction (Humphrey *et al.*, 1990; Friberg *et al.*, 1991; MacIntyre *et al.*, 1993; Humphrey and Goadsby, 1994), is able to induce antinociception by increasing cerebral ACh release, we carried out various common analgesic tests and determined hippocampal ACh extracellular levels in laboratory animals.

Received for publication August 17, 1995.
[1] This research was supported by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST)(CNR-F.A.T.M.A.).

ABBREVIATIONS: 8-OH-DPAT, 8-hydroxy-2-di-n-propylamino-tetralin; HC-3, hemicholinium-3; 5-HT, 5-hydroxytryptamine; NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine; CGP-35348, 3-aminopropyl-diethoxy-methyl-phosphinc acid; SDZ-205557, 2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester.

Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

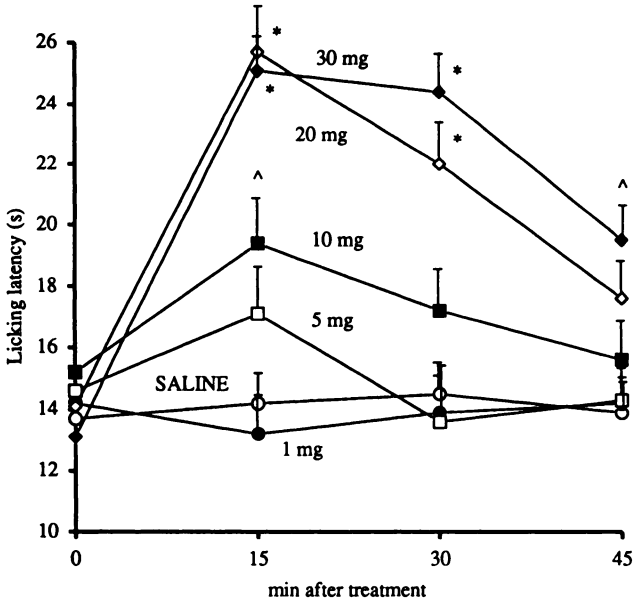


Fig. 1. Dose-response curves of sumatriptan in the mouse hot-plate test. The doses are expressed as mg · kg^{-1} i.p. Vertical lines show S.E.M. ^ P < .05; * P < .01 compared with saline controls. Each point represents the mean of at least 10 mice.

Materials and Methods

Animals. Male Swiss-Webster mice (22–28 g), male Wistar rats (120–200 g) from Morini (San Polo d'Enza, Italy), male Sprague-Dawley rats (220–250 g) from Charles River (Calco, Italy) and female guinea pigs (150–200 g) from Rodentia (Bergamo, Italy) were used. The animals were kept at 23°C ± 1°C, with a 12-h light/dark cycle, light at 7 A.M., and had food and water *ad libitum*. All experiments were carried out according to the guidelines of the European Community Council on animal care.

Rota-rod. The integrity of motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod in accordance with Vaught *et al.* (1985). The performance time was measured before treatment and 15, 30 and 45 min after treatment.

Hot-plate test. We adopted the method described by O'Callaghan and Holtzman (1976), using a stainless steel container (36 × 28 × 30 cm), thermostatically set at 52.5°C ± 0.1°C, in a precision water bath. Mice with a licking latency below 12 and over 18 s in the test before drug administration (30%) were rejected. An arbitrary cutoff time of 45 s was adopted.

Abdominal constriction test. The test was performed in mice in accordance with Koster *et al.* (1959). The number of stretching movements was counted for 10 min, starting 5 min after 0.6% acetic acid injection.

Paw-pressure test. The nociceptive threshold in the Wistar rats and guinea pigs was determined with an analgesimeter (Ugo Basile, Varese, Italy), according to the method described by Leighton *et al.* (1988). Rats and guinea pigs that scored below 40 g or over 80 g during the test before drug administration were rejected. An arbitrary cutoff value of 250 g was adopted.

Determination of ACh extracellular levels by cerebral microdialysis. Sprague-Dawley rats were anesthetized with chloral hydrate (0.4 g/kg i.p.) and implanted with a dialysis tube (AN 69-HF, wet tube outer diameter 320 μm; Hospal-Dasco, Bologna, Italy) at the level of dorsal hippocampus (A = −3 from bregma; V = −3.5 from skull) according to the König and Klippel atlas (König and Klippel, 1963). Surgery was carried out by using the transversal microdialysis technique, recently revised in order to cause less tissue damage and reduce the glia reaction around the dialysis tube (Imperato *et al.*, 1992). Ringer's solution containing (mM) KCl 3, NaCl 125, $CaCl_2$ 1.3, $MgCl_2$ 1.0, $NaHCO_3$ 23 and aqueous potassium phosphate buffer 1.5, pH 7.3, was pumped through the dialysis probe at a constant rate of 2 μl/min. To achieve a detectable amount of ACh in the dialysate, neostigmine 10^{-7} M was added to the Ringer's solution. The extracellular concentration of ACh was estimated in 20-min samples (40 μl) of dialysate by high-performance liquid chromatography with electrochemical detection according to the technique described by Damsma and Westerink (1991). The detection limit for ACh was 0.05 pmol per injection. Experiments were started 24 h after implantation of the dialysis tube. Values of ACh after saline or sumatriptan injection are expressed as percent of basal ACh extracellular concentrations. Each data point represents the mean ± S.E.M. of at least eight rats. Basal values for each rat were calculated by taking the mean of three or four samples that were not different from each other by more than 10%.

Drugs. The following drugs were used: atropine sulfate and physostigmine sulfate (Sigma), hemicholinium-3 hydrobromide, naloxone hydrochloride, NAN-190 hydrobromide and ketanserine tartrate (RBI), CGP-35348, clomipramine hydrochloride (Anafranil; Ciba-Geigy), sumatriptan succinate (Glaxo). SDZ-205557 ethyl ester hydrochloride was prepared in the Department of Pharmaceutical Sciences of Florence according to the method described by Romanelli *et al.* (1993). Other chemicals were of the highest quality commercially available. The doses given in the text are expressed as salts. All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared so that the necessary dose could be injected into a volume of 10 ml · kg^{-1} by both s.c. and i.p. routes. Intracerebroventricular administration was performed under short ether anesthesia according to the method described by Haley and McCormick (1957) for mice, which we adapted for rats. In mice and rats (rat coordinates are reported in parenthe-

TABLE 1
Effect of physostigmine on sumatriptan antinociception in the mouse hot-plate test

Treatment	Licking Latency (s)			
	Before Pretreatment	After Treatment		
		15 min	30 min	45 min
Saline	13.9 ± 0.9 (8)	14.3 ± 1.6 (8)	14.6 ± 1.2 (8)	13.8 ± 1.5 (8)
Physostigmine 0.05 mg/kg i.p.	13.7 ± 1.1 (15)	16.7 ± 1.5 (15)	14.1 ± 1.2 (15)	13.6 ± 1.4 (15)
Sumatriptan 20 mg/kg s.c.	14.1 ± 1.3 (8)	26.2 ± 2.2* (8)	21.9 ± 2.6^ (8)	17.5 ± 1.8 (8)
Physostigmine and sumatriptan	14.3 ± 0.7 (10)	37.2 ± 3.1*° (10)	28.5 ± 2.7*° (10)	25.4 ± 1.9*° (10)

The number of mice is shown in parentheses. Physostigmine was injected 5 min before sumatriptan. ^ P < .05; * P < .01 compared with saline controls. ° P < .05 *vs.* sumatriptan-treated mice.

Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

aspet

TABLE 2

Effects of atropine, HC-3, naloxone, CGP-35348, NAN-190, ketanserine, SDZ-205557 and quinpirole on sumatriptan antinociception in the mouse hot-plate test

Pretreatment	Treatment	mg · kg^{-1} i.p.	Licking Latency (s)			
			Before Pretreatment	After Treatment		
				15 min	30 min	45 min
Saline 10 ml · kg^{-1} i.p.	Saline		13.7 ± 0.6 (22)	14.2 ± 0.8 (22)	14.5 ± 0.9 (22)	13.9 ± 0.9 (22)
Saline 5 μl i.c.v.	Saline		13.9 ± 0.4 (29)	14.7 ± 0.9 (29)	14.3 ± 0.8 (29)	14.1 ± 1.1 (29)
Saline i.p. or i.c.v.	Sumatriptan	20	14.1 ± 0.6 (23)	25.7 ± 2.1** (23)	22.0 ± 1.5** (23)	17.6 ± 1.4 (23)
	Sumatriptan	30	13.1 ± 1.0 (20)	25.1 ± 1.5** (20)	24.4 ± 1.2** (20)	19.5 ± 1.3* (20)
Atropine 5 mg · kg^{-1} i.p.	Saline		14.8 ± 0.6 (23)	13.9 ± 0.9 (23)	14.1 ± 0.5 (23)	14.8 ± 0.8 (23)
	Sumatriptan	20	13.9 ± 1.2 (15)	15.7 ± 1.9^ (15)	15.8 ± 2.2^ (15)	16.1 ± 1.6 (15)
	Sumatriptan	30	14.1 ± 1.1 (12)	20.6 ± 1.7*^ (12)	19.9 ± 2.0* (12)	14.5 ± 1.9 (12)
HC-3 1 μg per mouse i.c.v.	Saline		13.7 ± 1.8 (26)	13.5 ± 0.8 (26)	16.2 ± 0.6 (26)	15.3 ± 0.7 (26)
	Sumatriptan	20	13.8 ± 1.1 (13)	14.0 ± 1.9^ (13)	13.6 ± 1.9^ (13)	13.0 ± 1.3 (13)
Naloxone 1 mg · kg^{-1} i.p.	Saline		13.8 ± 0.4 (15)	14.4 ± 0.7 (15)	13.4 ± 0.9 (15)	15.0 ± 0.7 (15)
	Sumatriptan	20	13.6 ± 0.5 (12)	25.1 ± 2.9** (12)	24.0 ± 2.2** (12)	17.3 ± 1.7 (12)
CGP 35348 100 mg · kg^{-1} i.p.	Saline		13.6 ± 0.7 (8)	11.6 ± 0.9* (8)	12.7 ± 1.1 (8)	12.3 ± 1.0 (8)
	Sumatriptan	20	13.7 ± 0.5 (8)	24.5 ± 1.5** (8)	18.7 ± 1.5 (8)	16.6 ± 1.3 (8)
NAN-190 0.5 mg · kg^{-1} i.p.	Saline		15.6 ± 1.4 (8)	15.1 ± 2.1 (8)	16.3 ± 2.1 (8)	13.7 ± 1.8 (8)
	Sumatriptan	20	14.4 ± 0.9 (10)	16.7 ± 1.6^ (10)	14.9 ± 1.7^ (10)	13.6 ± 1.8 (10)
Ketanserine 0.5 mg · kg^{-1} i.p.	Saline		14.1 ± 1.0 (10)	15.1 ± 1.8 (10)	13.7 ± 2.1 (10)	13.9 ± 1.3 (10)
	Sumatriptan	20	13.6 ± 1.1 (12)	28.0 ± 2.2** (12)	26.5 ± 1.4* (12)	18.9 ± 1.6 (12)
SDZ-205557 10 mg · kg^{-1} i.p.	Saline		13.7 ± 1.1 (10)	13.2 ± 1.9 (10)	14.1 ± 1.7 (10)	14.4 ± 1.5 (10)
	Sumatriptan	20	13.9 ± 0.9 (10)	26.1 ± 1.7** (10)	21.6 ± 2.0* (10)	17.6 ± 1.8 (10)

The number of mice is shown in parentheses.
** $P < .01$; * $P < .05$ compared with saline-saline; ^ $P < .01$ *vs.* saline-sumatriptan.

ses), hypodermic needle a 0.5 mm in external diameter attached to a 10-μl syringe was inserted perpendicularly through the skull and no more than 2 (4) mm into the brain, where 5 (10)-μl solution was injected. The injection site was 1.5 (2.5) mm from either side of the midline on a line drawn through the anterior base of the ears. To ascertain the exact site of i.c.v. injection, some mice and rats were injected i.c.v. with 5 (10) μl of 1:10 diluted 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 Fisher's PLSD procedure for *post-hoc* comparison, was used to verify significance between two means. P-values of less than .05 were considered significant. Data were analyzed with the StatView for the Macintosh computer program (1992).

Results

Effect on pain threshold. The antinociceptive effect of sumatriptan was investigated with the hot-plate test and abdominal constriction test in mice and the paw-pressure test in rats and guinea pigs. In the hot-plate test, sumatriptan injected i.p. at doses between 10 and 30 mg · kg^{-1}, induced a significant increase in the pain threshold (fig. 1). The antinociceptive effect reached a maximum 15 min after administration and then diminished, disappearing within 45 min. Table 1 shows that the antinociception induced by sumatriptan (20 mg · kg^{-1} s.c.) is significantly increased and prolonged by a physostigmine dose (0.05 mg · kg^{-1} i.p.) that by itself neither modifies the pain threshold nor exhibits cholinomimetic symptoms. Table 2 shows that sumatriptan antinociception was completely prevented by atropine (5 mg · kg^{-1} i.p.), by the choline uptake blocker HC-3 (1 μg per mouse i.c.v.) and by the 5-HT$_{1A}$ antagonist NAN-190 (0.5 mg · kg^{-1} i.p.), all injected 15 min before sumatriptan with the exception of HC-3, which was administered 5 h before the analgesic test. Conversely, no modification in sumatriptan antinociception was obtained by pretreating the mice with the opioid antagonist naloxone (1 mg · kg^{-1} i.p.), the GABA$_B$ antagonist CGP-35348 (100 mg · kg^{-1} i.p.), the 5-HT$_2$ antagonist ketanserine (0.5 mg · kg^{-1} i.p.) or the 5-HT$_4$ antagonist SDZ-205557 (10 mg · kg^{-1} i.p.). CGP-35348 was injected 5 min before sumatriptan, and naloxone, ketanserine and SDZ-205557 were injected 15 min before sumatriptan administration.

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

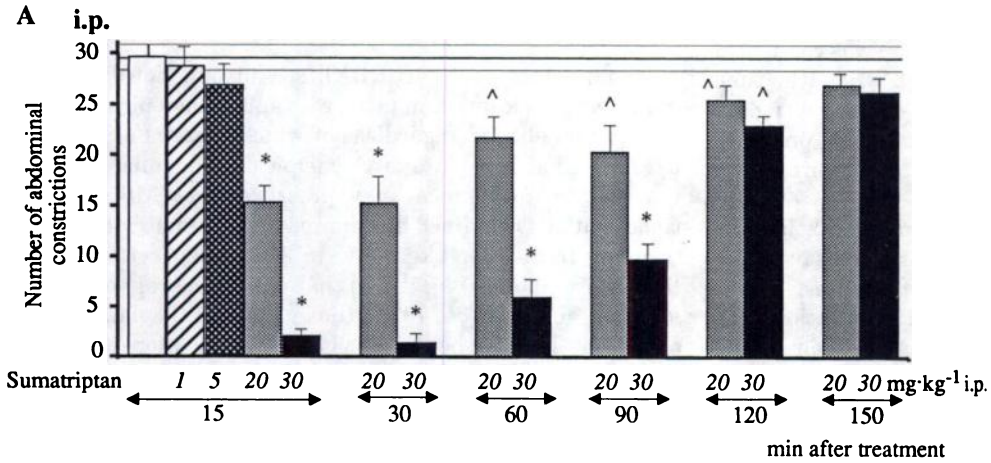
Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

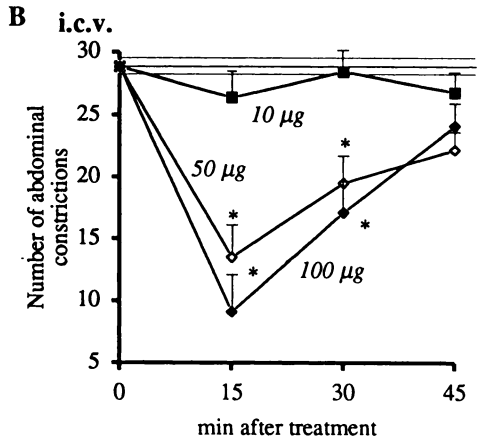
TABLE 3

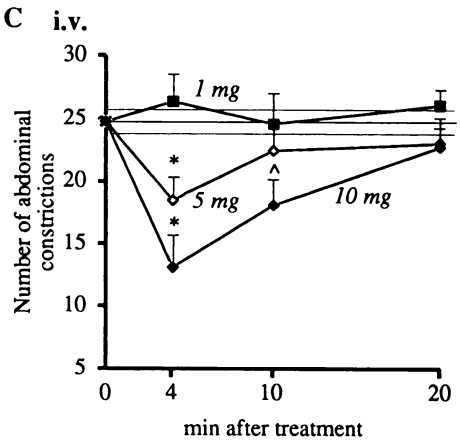
Effect of sumatriptan injected i.c.v. in the mouse hot-plate test

Treatment (i.c.v.)	Dose (μg per mouse)	Licking Latency (s)			
		Before Treatment	After Treatment		
			15 min	30 min	45 min
Saline		13.9 ± 0.9	15.0 ± 2.0	14.9 ± 1.7	14.0 ± 1.9
Sumatriptan	1	14.0 ± 0.9	13.6 ± 2.1	14.1 ± 1.8	14.0 ± 2.7
Sumatriptan	5	13.6 ± 1.1	14.1 ± 2.0	14.6 ± 2.1	14.9 ± 1.9
Sumatriptan	10	14.5 ± 1.1	16.8 ± 2.1	16.7 ± 1.8	15.4 ± 1.3
Sumatriptan	50	13.9 ± 0.9	20.5 ± 1.3*	21.2 ± 1.3*	15.8 ± 1.2
Sumatriptan	100	14.2 ± 1.3	24.6 ± 1.3*	21.4 ± 1.3*	17.1 ± 1.2

The number of mice ranged from 10 to 20.
* P < .01 compared with saline controls.







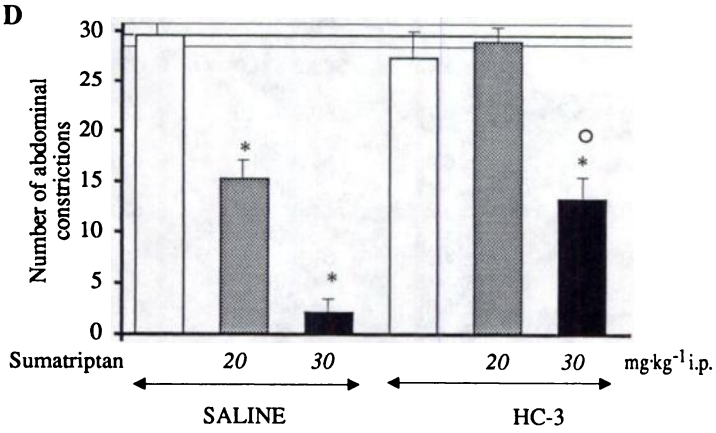


Fig. 2. Dose-response curves of sumatriptan administered i.p. (A), i.c.v. (B) and i.v. (C) in the mouse abdominal constriction test. (D) Effect of HC-3 (1 μg per mouse i.c.v.) pretreatment on antinociception induced by sumatriptan. HC-3 was injected 5 h before the test. Sumatriptan in panel D was injected 15 min before the test. Vertical lines show S.E.M. ^P < .05; * P < 0.01 compared with saline controls. ° P < .05 compared with sumatriptan (30 mg · kg^{-1} i.p.). Each column represents the mean of at least eight mice.

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

Table 3 shows the results obtained on the hot-plate test in the mouse after i.c.v. administration of sumatriptan. The antinociception obtained was of comparable intensity to that observed after i.p. injection.

The dose-response curves of sumatriptan administered i.p., i.c.v. and i.v. on the abdominal constriction test are shown in figure 2 (panels A, B and C). Sumatriptan, injected i.p. at the doses of 20 and 30 mg · kg^{-1}, induced antinociception that lasted 2h (fig. 2A). Both doses decreased the number of abdominal constrictions and had a maximum effect 15 and 30 min after treatment. Likewise, sumatriptan, injected i.c.v. at 50 to 100 μg per mouse and i.v. at 5 to 10 mg · kg^{-1} induced antinociception (fig. 2, B and C). HC-3 prevented the antinociceptive effect of sumatriptan at the dose of 20 mg · kg^{-1} i.p. but did not completely block antinociception induced by sumatriptan at the dose of 30 mg · kg^{-1} i.p. (fig. 2D). As shown in table 4, sumatriptan antinociception was confirmed in the rat and guinea pig paw-pressure test, where the time course reflected that observed with the hot-plate test (fig. 1). In the paw-pressure test, as in the hot-plate test, pretreatment of rats with atropine or with HC-3 prevented sumatriptan antinociception.

Finally, it should be noted that sumatriptan elicited its antinociceptive effect without changing either gross behavior or motor coordination as revealed by the rota-rod test, where sumatriptan, administered within the antinociception dose range, did not increase the number of falls from the rotating rod in comparison with saline treated mice (table 5). The number of falls in the rota-rod test progressively decreased as a function of the number of experimental sessions. In other words, mice progressively learned to remain in equilibrium on the rotating rod.

Effect on ACh extracellular levels. The basal hippocampal ACh recovered by freely moving rats was 3.04 ± 0.07 pmol/20 min (mean ± S.E.M.; 26 rats); in control animals, this amount remained stable throughout the experiment. As shown in figure 3, sumatriptan dose-dependently brought about a statistically significant increase in dialyzed ACh, which peaked 40 to 60 min after administration and returned to basal values within 120 min. The maximum percentage increases in recovered ACh were 149.3 ± 3.0% and 208.0 ± 2.1%, respectively, for the doses of 15 and 30 mg · kg^{-1} i.p.

Discussion

Sumatriptan, like 8-OH-DPAT (see introduction), is able to induce antinociception in mice, rats and guinea pigs. Antinociception is elicited regardless of which noxious stimulus is used: thermal (hot-plate test), chemical (abdominal constriction test) or mechanical (paw-pressure test). Although antinociception is obtained by administering sumatriptan doses (ranging from 5 mg · kg^{-1} i.v. to 30 mg · kg^{-1} i.p.) higher than those (0.5 mg · kg^{-1} i.v.) that elicit cerebral vessel constriction (Kobari *et al.*, 1993), the increase in the pain threshold is obtained without any visible modification of animal behavior (the researchers, who were unaware of the treatment received by the animals, were unable to distinguish between controls and sumatriptan-treated groups). More-

TABLE 4

Antinociception exerted by sumatriptan in the paw-pressure test: in the rat (A) and the guinea pig (B) and antagonism by atropine and HC-3 on sumatriptan antinociception in rats

Pretreatment	Treatment (i.p.)	Dose mg · kg^{-1}	Paw Pressure (g)			
			Before Pretreatment	After Treatment		
				15 min	30 min	45 min
A) Saline 10 ml · kg^{-1} i.p.	Saline		64.9 ± 3.2 (12)	63.0 ± 3.0 (12)	60.4 ± 3.6 (12)	62.0 ± 3.0 (12)
Saline 10 μl i.c.v.			63.2 ± 3.8 (9)	66.5 ± 3.2 (9)	64.6 ± 3.0 (9)	61.5 ± 3.2 (9)
Saline i.p. or i.c.v.	Sumatriptan	10	65.6 ± 4.0 (5)	68.0 ± 4.2 (5)	62.0 ± 4.0 (5)	55.8 ± 5.4 (5)
	Sumatriptan	20	65.0 ± 3.6 (10)	114.8 ± 5.0* (10)	90.0 ± 4.2* (10)	64.5 ± 4.0 (10)
	Sumatriptan	30	64.0 ± 4.0 (8)	120.6 ± 6.2* (8)	95.0 ± 4.2* (8)	70.5 ± 5.0 (8)
Atropine 5 mg · kg^{-1} i.p.	Saline		58.8 ± 3.0 (10)	62.2 ± 4.0 (10)	60.6 ± 3.2 (10)	60.2 ± 4.0 (10)
	Sumatriptan	20	62.8 ± 2.8 (5)	66.2 ± 5.4^ (5)	63.2 ± 4.0^ (5)	62.1 ± 4.8 (5)
HC-3 1 μg per rat i.c.v.	Saline		63.8 ± 4.0 (10)	63.7 ± 4.7 (10)	60.2 ± 3.0 (10)	58.4 ± 4.4 (10)
	Sumatriptan	20	64.0 ± 4.2 (5)	56.0 ± 6.4^ (5)	56.0 ± 4.0^ (5)	62.0 ± 6.4 (5)
B)	Saline		62.3 ± 3.0 (4)	56.8 ± 5.2 (4)	54.9 ± 5.8 (4)	52.4 ± 5.5 (4)
	Sumatriptan	1	64.8 ± 4.0 (5)	59.6 ± 4.0 (5)	57.8 ± 4.2 (5)	53.8 ± 4.8 (5)
	Sumatriptan	10	60.2 ± 4.8 (6)	68.8 ± 5.6 (6)	66.8 ± 4.4 (6)	52.8 ± 5.0 (6)
	Sumatriptan	20	58.0 ± 3.2 (5)	102.0 ± 4.3* (5)	82.0 ± 4.0* (5)	66.8 ± 3.2 (5)

The numbers of rats and guinea-pigs are shown in parentheses.
* $P < .01$ compared with saline-saline; ^ $P < .01$ *vs.* saline-sumatriptan.

Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

TABLE 5
Effect of sumatriptan in the mouse rota-rod test

Treatment	Dose	Number of Falls (30s)			
		Before Treatment	After Treatment		
			15 min	30 min	45 min
Saline		2.2 ± 0.3	1.8 ± 0.2	1.4 ± 0.2^	0.6 ± 0.2^^
Sumatriptan	10 mg · kg^{-1} i.p.	2.4 ± 0.2	2.1 ± 0.4	1.3 ± 0.3^	0.9 ± 0.2^^
Sumatriptan	20 mg · kg^{-1} i.p.	2.3 ± 0.4	1.7 ± 0.3	1.1 ± 0.3^^	0.8 ± 0.3^^
Sumatriptan	30 mg · kg^{-1} i.p.	2.4 ± 0.4	1.9 ± 0.2	1.3 ± 0.3^	0.7 ± 0.2^^
Sumatriptan	50 mg · kg^{-1} i.p.	2.1 ± 0.3	2.4 ± 0.3*	2.2 ± 0.3*	1.9 ± 0.2*
Saline	5 μl i.c.v.	2.1 ± 0.4	1.6 ± 0.3	1.5 ± 0.2^	1.1 ± 0.1^^
Sumatriptan	100 μg i.c.v.	2.3 ± 0.5	1.9 ± 0.3	1.6 ± 0.3^	1.3 ± 0.3^^
Sumatriptan	150 μg i.c.v.	2.5 ± 0.4	2.6 ± 0.2*	2.0 ± 0.3*	1.9 ± 0.4*

Each value represents the mean of 10 mice.
* P < .01 compared with saline controls.
^ P < .05, ^^ P < .01 compared with the respective pretest value.

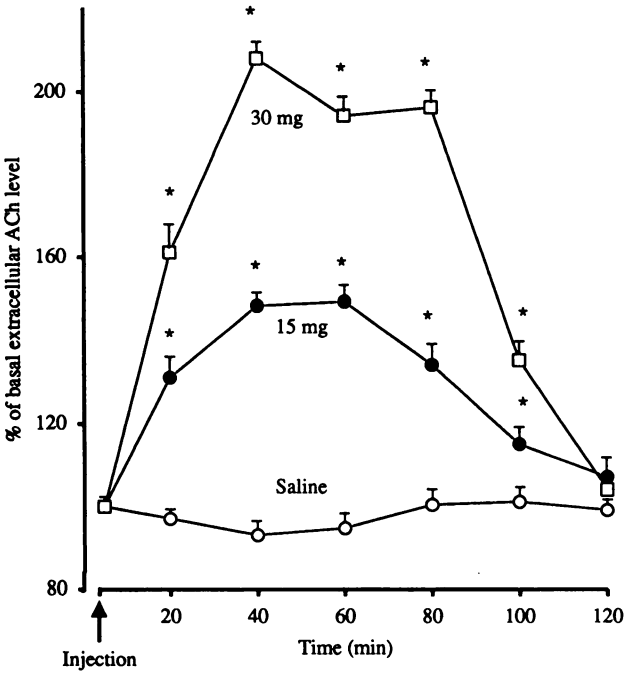


Fig. 3. Dose-response curves of sumatriptan on extracellular ACh level from rat hippocampus. The doses are expressed as mg · kg^{-1} i.p.. Vertical lines give S.E.M. Each point represents the mean of at least 8 to 10 independent experiments. * P < .01 compared with saline controls.

over, the sumatriptan-treated mice exhibit a normal performance on the rota-rod test.

For the following reasons, we know that sumatriptan exerts its antinociceptive effect by acting centrally. 1) It is possible to reach the same intensity of analgesia by injecting, directly into the cerebral ventricles, doses (50–100 μg per mouse) of sumatriptan that are considerably lower than those needed parenterally. Dependence of the antinociception on a retrodiffusion of the drug from the cerebral ventricles to the periphery can thus be ruled out. 2) The i.c.v. administration of HC-3 is able to antagonize the increase in the sumatriptan pain threshold.

Sumatriptan antinociception is dependent on central cholinergic activation and 5-HT$_{1A}$ receptor stimulation. Sumatriptan antinociception is prevented not only by the 5-HT$_{1A}$ antagonist NAN-190 but also by the muscarinic antagonist atropine and the ACh depletor HC-3. Therefore, the effectiveness of the central cholinergic system is fundamental for sumatriptan antinociception. It is well known that direct or indirect cholinomimetics are able to increase the pain threshold in both humans (Hood *et al.*, 1995) and animals (George *et al.*, 1962; Herz, 1962; Ireson, 1970; Bartolini *et al.*, 1987 and 1992).

Other neurotransmitter systems are not involved in sumatriptan antinociception; the opioid antagonist naloxone, the GABA$_B$ antagonist CGP-35348, the 5-HT$_2$ antagonist ketanserine and the 5HT$_4$ antagonist SDZ-205557 are all unable to prevent the sumatriptan effect. The doses and administration schedules of the above-mentioned drugs are suitable for preventing antinociception induced by morphine (Ghelardini *et al.*, 1990), GABA$_B$ agonists (Malcangio *et al.*, 1991) and 5HT$_4$ agonists (Ghelardini *et al.*, 1993). As far as ketanserine is concerned, we used the highest dose that is devoid of effect on locomotor activity. This was necessary because no selective 5-HT$_2$ agonists were available to enable us to determine the exact antagonistic dose of ketanserine. Moreover, the lack of availability of selective antagonists for 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors prevented us from ruling out the possible involvement of these two receptors. The fact that other 5-HT$_{1A}$ agonists, such as 8-OH-DPAT (Ghelardini *et al.*, 1994), buspirone and gepirone (Ghelardini *et al.*, 1995), are able to induce antinociception of a cholinergic type, just as sumatriptan does, seems to confirm the conclusion that 5-HT$_{1A}$ receptors play a role, but it does not rule out the possibility that other 5-HT receptor subtypes may also be involved.

Our results indicate a good relationship between the antinociceptive effect of sumatriptan and the increase of hippocampal ACh levels. Because the brain structures involved in sumatriptan antinociception are as yet unknown, we had no criteria for selecting a specific area for measuring ACh release through microdialysis experiments. We chose the hippocampus not only because it contains a high density of 5-HT$_{1A}$ receptors (Desmukh *et al.*, 1983; Verge *et al.*, 1986), but also because the activation of 5-HT$_{1A}$ receptors in this structure caused an increase of ACh release that was blocked by NAN-190 (Izumi *et al.*, 1994). Moreover, the hippocampus is very rich in muscarinic M$_1$ receptors (Mash and Potter, 1986), which are responsible for cholinergic-type antinociception (Bartolini *et al.*, 1992).

The greater latency required to reach the maximum increase in ACh concentration (40 min) compared with that required for the antinociceptive peak (15 min) could be ascribed to the time taken by ACh to diffuse from the synaptic cleft to the microdialysis tube. Moreover, we must remember

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet
Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012

that microdialysis experiments were performed in the presence of physostigmine, which, by inhibiting the degradation of ACh, potentiates and prolongs the increase of extracellular ACh due to sumatriptan. This could explain the discrepancy between the time courses of sumatriptan antinociception, obtained in the absence of physostigmine, and of ACh levels measured in microdialysis experiments. As a matter of fact, sumatriptan antinociception is greatly potentiated and prolonged by a dose of physostigmine that by itself is devoid of effect (table 1).

Skingle *et al.* (1990) have reported that sumatriptan has little or no antinociceptive activity against a range of noxious stimuli in rodents. Because the doses of sumatriptan injected by these authors were in the same range as ours, the striking discrepancy between our and their results is probably due to the excessive delay with which these authors detected the pain threshold. Unfortunately, Skingle *et al.* (1990) do not report the time elapsed from sumatriptan injection to revelation of the pain threshold. Our data show that the sumatriptan antinociceptive effect almost disappears after 30 min. At this time, morphine reaches its maximum analgesic activity. Because Skingle *et al.* compared the effect of sumatriptan with morphine's antinociceptive effect, we suppose that they used the same experimental parameters for both drugs, which may have impeded the disclosure of sumatriptan antinociception.

In summary, our results show that sumatriptan is able to potentiate endogenous cholinergic activity, which may account for at least some reported side effects elicited by the drug during migraine therapy. It remains to be determined whether the sumatriptan antinociception observed in rodents and guinea pigs contributes to the antimigraine activity elicited by the drug in humans.

Acknowledgments

The authors wish to thank Glaxo for the generous gift of sumatriptan. Preliminary data were presented at the XII IUPHAR Congress, Montreal, July 24 to 29, 1994.

References

Bartolini, A., Galli, A., Ghelardini, C., Giotti, A., Malcangio, M., Malmberg-Aiello, P. and Zucchi, P. L.: Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism. Br. J. Pharmacol. **92:** 711–721, 1987.

Bartolini, A., Ghelardini, C., Fantetti, L., Malcangio, M., Malmberg-Aiello, P. and Giotti, A.: Role of muscarinic receptor subtypes in central antinociception. Br. J. Pharmacol. **105:** 77–82, 1992.

Bianchi, C., Siniscalchi, A. and Beani, L.: 5-HT_{1A} agonists increase and 5-HT_3 agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs. Br. J. Pharmacol. **101:** 448–452, 1990.

Damsma, G. and Westerink, B. H. C.: A microdialysis and automated on-line analysis approach to study central cholinergic transmission *in vivo*. *In* Microdialysis in Neurosciences, ed. by T. E. Robinson and J. B. Justice, pp. 237–252, Elsevier, Amsterdam, 1991.

Dechant, K. L. and Clissold, S. P.: Sumatriptan—a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the acute treatment of migraine and cluster headache. Drugs **43 (5):** 776–798, 1992.

Desmukh, P. P., Yamamura, H. I., Woods, L. and Nelson, D. L.: Computer-assisted autoradiographic localization of subtypes of serotonin-1 receptors in rat brain. Brain Res. **288:** 338–343, 1983.

Doenicke, A., Brand, J. and Perrin, V. L.: Possible benefit of GR43175, a novel 5-HT_1-like receptor agonist, for the acute treatment of severe migraine. Lancet **i:** 1309–1311, 1988.

Friberg, L., Olesen, J., Iversen, H. K. and Sperling, B.: Migraine pain associated with middle cerebral artery dilatation: Reversal by sumatriptan. Lancet **338:** 13–17, 1991.

George, R., Haslett, W. L. and Jendel, D. J.: The central action of metabolite of tremorine. Life Sci. **1:** 361–363, 1962.

Ghelardini, C., Malmberg-Aiello, P., Giotti, A., Malcangio, M. and Bartolini, A.: Investigation into atropine-induced antinociception. Br. J. Pharmacol. **101:** 49–54, 1990.

Ghelardini, C., Giotti, A., Gualtieri, F., Matucci, R., Romanelli, M. N., Scapecchi, S., Teodori, E. and Bartolini, A.: Presynaptic auto- and heteroreceptors in the cholinergic regulation of pain. *In* Trends in Receptor Research, ed. by P. Angeli, U. Gulini and W. Quaglia pp. 95–114, Elsevier, Amsterdam, 1992.

Ghelardini, C., Fanfani, L., Galeotti, N., Giotti, A., Rizzi, A. and Bartolini, A.: 5-HT_4 agonists: BIMU-1 and BIMU-8 induce analgesia by facilitating central cholinergic transmission (Abstract). Soc. Neurosci. **19:** 565.2, 1378, 1993.

Ghelardini, C., Galeotti, N., Baldini, M., Meoni, P., Giotti, A. and Bartolini, A.: The 5-HT_{1A} agonist 8-OH-DPAT induces antinociception through a cholinergic mechanism. Can. J. Physiol. Pharmacol. **72:** suppl. 1, P13.9.43, 380, 1994.

Ghelardini, C., Galeotti, N., Fantetti, L. and Bartolini, A.: Buspirone and gepirone: Two anxiolytic drugs endowed with antinociception mediated by the cholinergic system. Behav. Pharmacol. **6:** suppl. 1, 42–43, 1995.

Haley, T. J. and McCormick, W. G.: Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. Chemother. **12:** 12–15, 1957.

Herz, A.: Wirkungen des Arecolins auf das Zentralnervensystem. Arch. Exp. Pathol. Pharmacol. **242:** 414–429, 1962.

Hood, D. D., Eisenach, J. C. and Tuttle, R.: Phase I safety assessment of intrathecal neostigmine methylsulphate in humans. Anesthesiology **82:** 331–343, 1995.

Humphrey, P. P. A., Feniuk, W., Perren, M. J., Motevalian, M. and Whalley, E. T.: The mechanism of anti-migraine action of sumatriptan. (Abstract) 2nd IUPHAR Satellite Meeting on Serotonin, p. 102. Basel, Switzerland, 1990.

Humphrey, P. P. A. and Feniuk, W.: Mode of action of the anti-migraine drug sumatriptan. TIPS **12:** 444–446, 1991.

Humphrey, P. P. A. and Goadsby, P. J.: The mode of action of sumatriptan is vascular? Cephalalgia **14:** 401–410, 1994.

Imperato, A., Angelucci, L., Zocchi, A., Casolini, P. and Puglisi-Allegra, S.: Repeated stressful experiences differently affect limbic dopamine release during and following stress. Brain Res. **577:** 194–199, 1992.

Ireson, J. D.: A comparison of the antinociceptive action of cholinomimetic and morphine-like drugs. Br. J. Pharmacol. **40:** 92–101, 1970.

Izumi, J., Washizuka, M., Miura, N., Hiraga, Y. and Ikeda, Y.: Hippocampal serotonin 5-HT_{1A} receptor enhances acetylcholine release in conscious rats. J. Neurochem. **62:** 1804–1808, 1994.

Kobari, M., Fukuuchi, Y., Tomita, M., Tanahashi, N., Konno, S. and Takeda, H.: Effects of sumatriptan on the cerebral intraparenchymal microcirculation in the cat. Br. J. Pharmacol. **110:** 1445–1448, 1993.

König, J. F. R. and Klippel, R. A.: The Rat Brain. The Williams and Wilkins Co., Baltimore, 1963.

Koster, R., Anderson, M. and De Beer, E. J.: Acetic acid for analgesic screening. Fed. Proc. **18:** 412, 1959.

Leighton, G. E., Rodriguez, R. E., Hill, R. G. and Hughes, J.: *K*-Opioid agonists produce antinociception after i.v. and i.c.v. but not intrathecal administration in the rat. Br. J. Pharmacol. **93:** 553–560, 1988.

Macintyre, P. D., Bhargava, B., Hogg, K. J., Gemmil, J. D. & Hillis, W. S.: Effect of subcutaneous sumatriptan, a selective 5HT_1 agonist, on the systemic, pulmonary and coronary circulation. Circulation **87 (2):** 401–405, 1993.

Malcangio, M., Ghelardini, C., Giotti, A., Malmberg-Aiello, P. and Bartolini, A.: CGP-35348, a new $GABA_B$ antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen. Br. J. Pharmacol. **103:** 1303–1308, 1991.

Mash, D. C. and Potter, L. T.: Autoradiografic localization of M_1 and M_2 muscarinic receptors in the rat brain. Neuroscience **19:** 551–564, 1986.

McCarthy, B. G. and Peroutka, S. J.: Comparative neuropharmacology of dihydroergotamine and sumatriptan (GR 43175). Headache 420–422, 1989.

Millan, J. M.: Serotonin and pain: Evidence that activation of 5-HT_{1A} receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. Pain **58 (1):** 45–61, 1994.

O'Callaghan, J. P. and Holtzman, S. G.: Prenatal administration of morphine to the rat: Tolerance to the analgesic effect of morphine in the offspring. J. Pharmacol. Exp. Ther. **197:** 533–544, 1976.

Romanelli, M. N., Ghelardini, C., Dei, S., Matucci, R., Mori, F., Scapecchi, S., Teodori, E., Bartolini, A., Galli, A., Giotti, A. and Gualtieri, F.: Synthesis and biological activity of a series of aryl tropanyl ester and amides chemically related to ICS 205930: Development of a 5-HT_4 agonist endowed with potent antinociceptive activity. Arzneim. Forsch/Drug Res., **43(II):** n°8, 913–918, 1993.

Skingle, M., Birch, P. J., Leighton, G. E. and Humphrey, P. P. A.: Lack of antinociceptive activity of sumatriptan in rodents. Cephalalgia **10 (5):** 207–212, 1990.

Vaught, J. L., Pelley, K., Costa, L. G., Setler, P. and Enna, S. J.: A comparison of the antinociceptive responses to GABA-receptor agonists THIP and baclofen. Neuropharmacology **24:** 211–216, 1985.

Verge, D., Davel, G., Marcinkiewicz, M., Patey, A., El Mestikawy, S., Gozlan, H. and Hamon M.: Quantitative autoradiography of multiple 5-HT_1 receptor subtypes in the brain of control or 5,7-dihy-droxytryptamine treated rats. J. Neurosci. **6:** 3474–3482, 1986.

Send reprint requests to: Prof. Alessandro Bartolini, Department of Pharmacology, University of Florence, viale G. B. Morgagni 65 - 50134 Firenze - Italy.

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

Downloaded from jpet.aspetjournals.org at Univ Studi Di Firenze on October 28, 2012