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Serotonergic Activity in Rat Cerebral Arteries Depends on Dorsal Raphe Nucleus but not on Median Raphe Nucleus

M. J. Moreno, A. L. López de Pablo, M. V. Conde, M. L. Fraile and E. J. Marco

Departamento de Fisiología,
Universidad Autónoma de Madrid, Madrid.

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The effect of Chlorimipramine and Muscimol on serotonergic activity in rat cerebral arteries and in dorsal and median raphe nuclei were used to study the presence of a serotonergic innervation in the cerebral blood vessels functionally dependent on the brainstem nuclei activity. Serotonergic activity was appraised in rat cerebral arteries from 5-hydroxyindoleacetic acid (5-HIAA) disappearance rate or 5-hydroxytryptophan (5-HTP) accumulation after inhibiting monoamine oxidase (MAO) or aromatic amino acids decarboxylase, respectively. In dorsal and median raphe nuclei the decay with time of 5-HIAA after MAO inhibition was used to estimate serotonergic activity. Chlorimipramine significantly reduced serotonergic activity in cerebral blood vessels and in both raphe nuclei. 5-HIAA basal levels in these blood vessels were not altered by treatment with the drug. Muscimol evoked only a decrease in the serotonergic activity of the median raphe nucleus. These results suggest that rat cerebral arteries receive serotonergic fibers functionally active arising mainly from dorsal raphe nucleus.

Key words: Cerebral arteries, Serotonin, Innervation, Raphe nuclei, Chlorimipramine, Muscimol.

There is increasing evidence, mostly biochemical and pharmacological, that supports the innervation of cerebral blood vessels by serotonergic fibers. Tryptophan or pargyline administration evokes an increase in their serotonin (5-HT) content whereas it appears diminished after inhibiting tryptophan hydroxylase (22, 25, 27). The presence of this enzyme has been shown *in vivo*, from the

Correspondence to Dr. E. J. Marco, Departamento de Fisiología, Universidad Autónoma de Madrid, Arzobispo Morcillo 2, 28029 Madrid (Spain), (Phone: 34-1-3975422. Fax: 34-1-3150075)

accumulation of 5-hydroxytryptophan after inhibiting aromatic amino acids decarboxylase (3, 27), and assayed *in vitro* (19). The innervation originates at least from dorsal and median raphe nuclei for the destruction of these nuclei brings about a decrease in 5-HT levels of the cerebral blood vessels (5, 15, 25) and a decrease in tryptophan hydroxylase activity (19). Since electrical stimulation of dorsal raphe nucleus evokes a regional decrease in the rat cerebral blood flow (1) and its lesion induces the development of supersensitivity to serotonin in the isolated middle cerebral artery of the cat (18), this innervation seems to be functionally active.

However, most of the morphological evidence, challenges the existence of such an innervation and favors that the serotonin detected in the cerebral blood vessels is just an artifact due to its uptake by the sympathetic nerve endings during the tissue isolation procedure (7, 8, 24, 28).

In the present report, the effects of chlorimipramine and muscimol on serotonergic activity in the cerebral arteries and in the dorsal and median raphe nuclei of the rat are studied. If changes specifically induced in serotonergic activity of cerebral blood vessels by these drugs can be related to alterations of the serotonergic activity in the raphe nuclei, this will lend support to the active presence in the cerebral blood vessels of a serotonergic innervation arising from those nuclei.

The 5-HT uptake blocker chlorimipramine (11), when given systemically to rats, decreases serotonergic activity in several brain areas including raphe nuclei (14, 17, 23, 26). There are no data regarding its effect on serotonergic nerve ending activity in the cerebral blood vessels.

Systemic administration of muscimol reduces 5-HT turnover in hippocampus but not in hypothalamus or in the cerebral arteries of the rat (16). Local injection of this drug in median and dorsal raphe nuclei evokes a decrease of serotonergic activity in the areas receiving projections from these nuclei (6, 21). However, little is known of its effect on these nuclei when administered systemically.

Materials and Methods

Male Sprague-Dawley rats, weighing 130-180 g, from the strain ICO:OFA SD (I.O.P.S. Caw) were used in the present study. The animals were housed in the proper facilities complying with the European Community directive 86/-609/CEE and Spanish legislation (R. D. 223/1988) regarding the care of the animals used in experimentation and other scientific purposes. The experiments reported here were approved by the Biosafety and Animal Care Unit Committee (Comisión de Bioseguridad y Gabinete Veterinario) of the University.

Serotonergic activity was appraised in cerebral blood vessels and brainstem nuclei from the rate of disappearance of 5-hydroxyindoleacetic acid (5-HIAA) after inhibiting monoamine oxidase (MAO) by pargyline (20, 27). In some experiments, the serotonergic activity in cerebral arteries was also estimated from the accumulation of 5-hydroxytryptophan (5-HTP) after administering benserazide, an aromatic amino acid decarboxylase inhibitor (27).

Some rats received chlorimipramine (20 mg/kg, i. p.) and were assorted in three different groups. The first one were killed by decapitation 15 minutes after being injected Chlorimipramine. The remaining two groups received pargyline (75 mg/kg, i. p.) instead of being killed and were sac-

rificed 30 and 60 minutes thereafter, respectively.

Other animals were injected muscimol (1 mg/kg i. v. through the tail vein) and also distributed in three groups. Forty minutes after Muscimol administration one group was sacrificed whereas the rest of the groups received pargyline (75 mg/kg, i. p.) and were subjected to the same schedule as the chlorimipramine-treated rats.

In some rats, benserazide (800 mg/kg, i. p.) was administered, 15 min after giving chlorimipramine or 45 min after injecting Muscimol, and the animals were decapitated 45 minutes later.

Each experimental group had its own control mates. These were given saline solution (1 ml/kg) injected through the same administration route and subjected to the same procedure as the corresponding drug-treated group. All the injected drugs were also dissolved in saline solution.

The doses and times of chlorimipramine and muscimol used were those found to be effective in the Central Nervous System (13, 14).

After sacrificing the rats by decapitation, the brain was quickly removed, the circle of Willis with some of its branches dissected out and the brain kept for later removal of the brainstem nuclei. All the tissues were frozen on dry ice and stored at –15 °C.

The frozen brain to be dissected was placed on a metal block cooled to –8 °C and 0.5–1 mm thick frontal slices were cut free hand with a razor blade. The slices were kept frozen on the block and the dorsal an median raphe nuclei punched out. The nuclei were identified following the description by KÖNIG and KLIPPEL (9).

The samples of the different tissues were sonicated in 0.4 M $HClO_4$ with 0.002 % (w/v) ascorbic acid and spun at 12,000 r.p.m. for 5 min. 5-HTP or 5-HIAA were assayed in 50 µl of the corresponding supernatants by high pressure liquid chromatography (HPLC) with electrochemical detection. Proteins in precipitates were determined by the method of LOWRY *et al.* (12), the final concentrations of the hydroxyindoles being referred to them. The HPLC system consisted of a Sample Injector (Gilson, model 231) with a dilutor (Gilson, model 401), a pump (Gilson, model 305) with a manometric module (Gilson, model 805), giving a flow rate of 1.200 ml/min, and a reverse phase column (µBondapak C_{18}, Waters) with a guard column (µBondapak C_{18}/Corasil, Waters). The hydroxyindoles were detected with an electrochemical detector (LC-4A, Bioanalytical Systems) and a glassy carbon electrode set at +0.5 V vs an Ag/AgCl reference electrode. The mobile phase was prepared according to LACKOVIC *et al.* (10).

In some animals, 5-HIAA was assayed in the whole blood. After decapitation, 4.8 ml of the collected blood were mixed with 2 ml of acid-citrate-dextrose solution (4). Two hundred µl of the mixture were added to 200 µl of a 0.8 M $HClO_4$ – 0.004 % ascorbic acid solution, sonicated and centrifuged. 5-HIAA was determined in the supernatant as indicated before.

The statistical analysis of the results was made by means of Student's *t* test.

Pargyline hydrochloride, 5-hydroxyindole-3-acetic acid, serotonin bitartrate, 5-hydroxy-L-tryptophan, and muscimol were purchased from SIGMA. Chlorimipramine hydrochloride was kindly gifted by Ciba and benserazide by Roche.

Results

The treatment of the animals with chlorimipramine (20 mg/kg, i. p.) induced a decrease in the value of the fractional rate constant (k) of 5-HIAA disappearance with time in cerebral arteries after inhibiting MAO with pargyline (fig. 1). No significant change was observed in 5-HIAA initial concentration of the chlorimipramine-treated animals brain blood vessels (13.64 ± 2.06 pmol/mg prot., n = 8) when compared to those from control (9.88 ± 1.77 pmol/mg prot., n = 8).

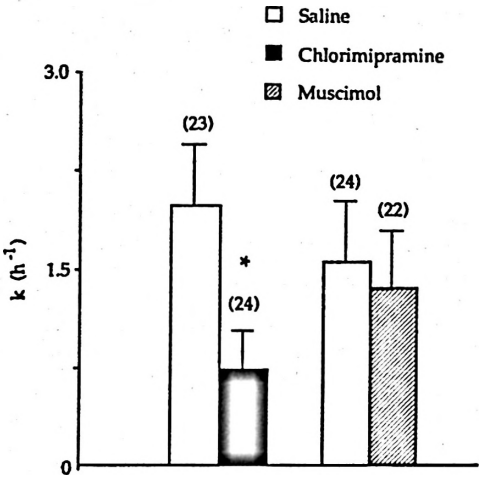

Fig 1. *Effect of Chlorimipramine (20 mg/kg, i.p.) and Muscimol (1 mg/kg, i.v.) on 5-HIAA decay fractional rate constant (k) in rat cerebral arteries.*
Control groups are saline-treated rats. Numbers in parentheses are total numbers of animals employed. Each vertical bar represents the mean value of k with S.E. of k from two pooled experiments. *p < 0.05.

Chlorimipramine also elicited a significant reduction in the accumulation of 5-HTP in brain base arteries after inhibition of the decarboxylase with benserazide (table I).

When muscimol (1 mg/kg, i. v.) was administered to the rats, no difference was found in the fractional rate constant of the decay with time of 5-HIAA (fig. 1). The initial values of the 5-HIAA content of the cerebral arteries were not significantly different from those found in the saline-treated animals (10.22 ± 2.07 pmol/mg prot., n = 8, and 7.36 ± 1.50 pmol/mg prot., n = 8, respectively).

Muscimol did not modify either 5-HTP accumulation after inhibition of aromatic amino acid decarboxilase (table I).

On the other hand, levels of 5-HIAA in whole blood of untreated animals were under the detection limit, which was 1 pmol/ml of blood.

The administration of chlorimipramine significantly decreased fractional rate constant of 5-HIAA decay in dorsal and median raphe nuclei (fig. 2). The initial levels of 5-HIAA were diminished ($p < 0.05$) by drug injection in the case of dorsal raphe nucleus (28.16 ± 4.24 pmol/mg prot., n = 8, in the drug-treated group vs 49.96 ± 8.57 pmol/mg prot., n = 7, in the saline-treated animals) whereas they remained unaffected in the median raphe nucleus (52.39 ± 4.18 pmol/mg prot., n = 8, and 60.22 ± 6.54 pmol/mg prot., n = 7, respectively).

Muscimol did not modify the fractional rate constant in dorsal raphe nucleus whereas it brought about a significant decrease of serotonergic activity in median raphe nucleus (fig. 2).

TABLE I. *Effect of Chlorimipramine (20 mg/kg, i.p.) and Muscimol (1 mg/kg, i.v.) on 5-HTP accumulation in rat cerebral arteries after aromatic amino-acids decarboxylase inhibition.*
Figures in parentheses are the number of animals used. Each value represents the mean values ± S.E.M. of two pooled experiments. *p < 0.05.

Treatment	5-HTP (pmol/mg prot.)	
Saline solution	8.30 ± 1.05	(8)
Chlorimipramine	4.80 ± 0.93*	(7)
Saline solution	11.74 ± 2.92	(7)
Muscimol	15.79 ± 3.09	(5)

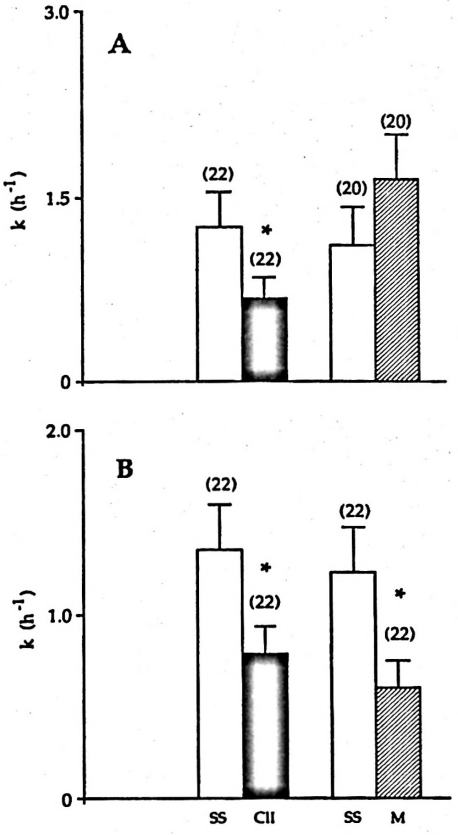

Fig. 2. *Effect of Chlorimipramine (ClI, 20 mg/kg, i.p.) and Muscimol (M, 1 mg/kg, i.v.) on 5-HIAA decay fractional rate constant (k) in rat dorsal (A) and median (B) raphe nuclei.*
Control animals received physiological saline solution (SS). Numbers in parentheses are total numbers of animals employed. Each vertical bar represents the mean value of k with S.E. of k from two pooled experiments. *$p < 0.05$.

Basal levels of 5-HIAA in dorsal raphe nucleus remained unaffected by the injection of the drug when compared with the saline-treated rats (64.36 ± 9.42 pmol/mg prot., n = 6, and 47.82 ± 8.40 pmol/mg prot., n = 8, respectively). A similar result was found in the median raphe nucleus: 82.29 ± 4.46 pmol/mg prot. (n = 7) in the animals receiving muscimol vs 75.62 ± 6.74 pmol/mg prot. (n = 8) in the control rats.

Discussion

The present data support the existence of a functional serotonergic innervation of rat brain base arteries whose main origin is, at least, dorsal raphe nucleus.

Chlorimipramine reduces serotonergic activity in rat cerebral arteries as deduced from its effect on fractional rate constant of 5-HIAA disappearance or on 5-HTP accumulation after aromatic amino acid decarboxilase inhibition. This action is similar to that exerted by chlorimipramine in other brain areas receiving serotonergic innervation (14). Since the drug also inhibits serotonergic activity in dorsal and median raphe nuclei, the serotonergic innervation ending in these vessels might originate from there. However, systemic administration of muscimol decreased serotonergic activity in median raphe nucleus without affecting it in cerebral blood vessels and dorsal raphe nucleus. These results, taken altogether, indicate that rat brain base blood vessels are innervated preferentially by dorsal raphe nucleus. This agrees with experiments showing that rat brain base arteries tryptophan hydroxylase activity diminishes after dorsal raphe destruction and remains unaltered after median raphe nucleus lesion (19), and that only the lesion of dorsal raphe nucleus, but not of median raphe nucleus, develops supersensitivity to 5-HT in the isolated middle cerebral artery of the cat (18).

Furthermore, the present results give additional evidence supporting the existence of serotonergic nerve endings in these vessels. Rat cerebral blood arteries contain 5-HIAA in spite of its levels being practically nil in blood. This 5-HIAA is the result of 5-HT metabolism in a structure with the ability to synthesize 5-HT rather than in sympathetic nerve endings

after taking the amine up, because pretreatment of the rats with chlorimipramine, a specific 5-HT uptake blocker, does not significantly reduce 5-HIAA levels in brain blood vessels, indicating that most of the serotonin has already been stored before the drug administration. These results are discordant with morphological evidence that challenges the existence of such an innervation and favors that the serotonin detected in the cerebral blood vessels by immunohistochemistry, is the result of its uptake by the sympathetic nerve endings during the tissue isolation procedure. Thus, 5-HT-like immunoreactivity falls dramatically when the blood vessels are perfused with saline solution, or the animals treated with an amine uptake blocker or 6-hydroxydopamine before dissecting the cerebral blood vessels (7, 8, 24, 28). The present results agree, however, with biochemical findings showing that perfusion of the vessels with saline solution before the sacrifice of the animals does not affect serotonin levels in rat cerebral vessels or that cervical gangliectomy does not abolish the presence of 5-HT in cerebral blood vessels (2, 5, 23)

In conclusion, cerebral arteries seem to receive a serotonergic innervation functionally active that can be manipulated pharmacologically. Chlorimipramine might be a tool to study the role of this innervation in cerebral circulation.

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M. J. MORENO, A. L. LÓPEZ DE PABLO, M. V. CONDE, M. L. FRAILE y E. J. MARCO. *La actividad serotonérgica en las arterias cerebrales de rata depende del núcleo dorsal del rafe, no del medial.* Rev. esp. Fisiol. (J. Physiol Biochem.), 50 (3), 175-182, 1994.

Se estudia el efecto de la clorimipramina y del muscimol sobre la actividad serotonérgica en arterias cerebrales de rata y en los núcleos dorsal y medial del rafe, para poner de manifiesto la presencia de una inervación serotoninérgica en los vasos cerebrales dependiente funcionalmente de la actividad de lo núcleos del tallo cerebral. La actividad serotonérgica se determina en las arterias cerebrales a partir de la velocidad de desaparición del ácido 5-hidroxi-indol-acético (5-HIAA) o de la acumulación de 5-hidroxitriptófano (5-HTP) tras inhibir la monoamino oxidasa (MAO) o la descarboxilasa de aminoácidos aromáticos, respectivamente. En los núcleos dorsal y medial del rafe se utiliza la caída del 5-HIAA con el tiempo tras inhibir la MAO como índice de la actividad serotonérgica. La clorimipramina reduce significativamente la actividad serotonérgica tanto en los vasos cerebrales como en ambos núcleos del rafe, sin alterar los niveles basales de 5-HIAA en los vasos. El muscimol disminuye de la actividad serotonérgica sólo en el núcleo medial del rafe. Estos resultados sugieren que las arterias cerebrales de la rata reciben fibras serotonérgicas funcionalmente activas con origen en el núcleo dorsal del rafe.

Palabras clave: Arterias cerebrales, Serotonina, Inervación, Núcleos del rafe, Clorimipramina, Muscimol.

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