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Simultaneous measurement of plasma protein extravasation and carotid vascular resistance in the rat

Rachel A. Spokes *, Vicki C. Middlefell

Dept. of Neuropharmacology, Wyeth Research (UK) Ltd., Huntercombe Lane South, Taplow, Berkshire SL6 0PH, UK

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Abstract

Stimulation of the right trigeminal ganglion in pentobarbital anaesthetised rats increased mean arterial blood pressure and decreased right carotid vascular resistance but had no effect on left carotid vascular resistance. Sumatriptan (0.3 mg/kg i.v.) pretreatment did not significantly affect basal levels or stimulation induced changes in blood pressure or carotid vascular resistance. Trigeminal stimulation produced plasma protein extravasation (measured using a fluorescent marker) into the dura mater on the ipsilateral side which was significantly reduced by sumatriptan. These studies show that sumatriptan can reduce plasma protein extravasation while having no measurable effect on total carotid blood flow.

Keywords: Sumatriptan; Plasma protein extravasation; Trigeminal ganglion stimulation; Dura mater; Carotid vascular resistance; Blood pressure

1. Introduction

Sumatriptan is an effective anti-migraine drug and a potent vasoconstrictor of intracranial blood vessels (Humphrey et al., 1990) which, it is thought, may be dilated during a migraine attack (Friberg et al., 1991). Sumatriptan also blocks plasma protein extravasation in the dura mater produced by trigeminal ganglion stimulation (Buzzi and Moskowitz, 1990). Since a sterile inflammation of the dura mater has also been implicated in the aetiology of migraine (Markowitz et al., 1987) this effect of sumatriptan may also be important for its anti-migraine action. The aim of this study was to measure carotid flow during stimulation of the trigeminal ganglion and to look at the effects of sumatriptan on carotid vascular resistance and plasma protein extravasation in the same animals to determine whether there may be a relationship between the two events.

Previous studies of plasma protein extravasation in the dura have utilised ^{125}I-labelled bovine serum albumin as a marker for extravasated protein (Markowitz et al., 1987). These authors also showed that Evans blue can be used a marker of plasma protein extravasation into larger extracranial tissues such as the lip and conjunctiva but this dye is not sufficiently fluorescent to be detected in the dura. Here we describe the use of fluoresceinisothiocyanate-bovine serum albumin (fluorescein) as an alternative marker for plasma protein extravasation which is sufficiently fluorescent (especially under alkaline conditions) to be measured in the dura mater as well as other extracranial tissues thus eliminating the need to use a radioactive marker in these studies.

2. Materials and methods

2.1. Evaluation of fluorescein as a marker for plasma protein extravasation

Male rats (270–350 g) were anaesthetised with pentobarbital sodium (70 mg/kg, i.p., on the midline). The left jugular vein was cannulated for marker injection and the abdomen shaved. Rats received intradermal injections of saline (0.1 ml/kg), and histamine (10 and 100 μg/kg) each in duplicate. The position of each injection was marked with a non-fluorescent

* Corresponding author. Tel. 01628 604377 ext. 4235, fax 01628 666507.

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marker pen. Rats were then injected with either filtered Evans blue (50 mg/kg, i.v., $n = 2$) or fluorescein (10 mg/kg, i.v., $n = 2$). After 60 min the rats were killed by cervical dislocation and exsanguination, the abdominal skin removed, and a circle of skin taken from around each injection site using an 11 mm cork borer. The skin circles from rats which had received Evans blue were placed in 5 ml formamide and the skin circles from the rats which had received fluorescein were placed in saline (0.9%, adjusted to pH 9 with NaOH). All samples were incubated at 55° C for 22 h. Evans blue concentrations were measured using a spectrophotometer (Pharmacia) set at a wavelength of 620 nm and calibrated with solutions of Evans blue in formamide (0.1–10 μg/kg + formamide as blank). Fluorescein concentrations [fluorescein] were measured using a Perkin-Elmer LS-30 luminescence spectrometer with the excitation wavelength set at 490 nm and an emission wavelength of 520 nm using a 1/20 sample dilution. A calibration curve for the luminescence spectrometer was obtained using concentrations of fluorescein from 10 to 200 ng/ml (pH 9) and using saline (pH 9) as zero. In all experiments drugs were made up in normal saline adjusted to pH 6–7 if necessary and injected in a volume of 1 ml/kg unless otherwise stated.

2.2. Efects of sumatriptan on basal systemic and carotid haemodynamics

Experiments were performed to study the effect of cumulative doses of sumatriptan on mean arterial blood pressure, carotid flow and carotid vascular resistance in the anaesthetised rat. Male rats (280–330 g, $n = 4$) were anaesthetised with pentobarbitone sodium (70 mg/kg, i.p.) and the right femoral artery and vein cannulated for continual recording of mean arterial blood pressure and drug administration respectively. The right common carotid artery was disected free and fitted with a Transonic flow probe (1 mm). Cumulative doses of sumatriptan from 0.01 to 3 mg/kg were administered i.v. at 5 min intervals. The mean arterial blood pressure, carotid flow and carotid vascular resistance (calculated as mean arterial blood pressure/carotid flow and expressed as mmHg/ml/min) were measured 5 min after each injection of sumatriptan.

2.3. Trigeminal ganglion stimulation in anaesthetised rats

The method was adapted from that of Markowitz et al. (1987). Male Sprague-Dawley rats (280–350 g) were anaesthetised with sodium pentobarbitone (70 mg/kg, i.p.) and prepared as in 2.2 above except that flow probes were placed around both carotid arteries. Animals were injected with either sumatriptan (0.3 mg/kg, i.v., $n = 6$) or saline (1 ml/kg, i.v., $n = 6$) and placed in a stereotaxic frame with the incisor bar at −2.5 mm. Bipolar electrodes were lowered into the trigeminal ganglia (3.5 mm AP, ±3.0 mm L and 9.5 mm V from Bregma) and the animal injected with fluorescein (50 mg/kg, i.v.). 10 min after sumatriptan or vehicle administration the right trigeminal ganglion was stimulated for 3 min (1.2 mA, 5 ms duration, 5 Hz). Mean arterial blood pressure and carotid flow were monitored before and during stimulation and carotid vascular resistance calculated as above. Rats were then perfused with saline at 120 mmHg for 3 min via the left cardiac ventricle, the brain removed, the electrode position checked and the dura from each side excised, rinsed and incubated in 2 ml of saline (pH 11) for 18–24 h. Dura were not collected from rats which had visible blood clots on the skull base. The dura were blotted dry and weighed and the [fluorescein] in the incubation solution measured using a Perkin-Elmer LS-30 luminescence spectrometer. Results are expressed as the ratio of [fluorescein] (in ng/mg tissue) on the ipsilateral side compared with the contralateral side. Statistical comparisons were performed using a 3 factor nested analysis of variance and Student's t-test for paired data or unpaired data.

3. Results

3.1. Evaluation of fluorescein as a marker for plasma protein extravasation

The calibration curve for fluorescein on the LS-30 showed that the response was linear over the range tested with a slope of 1.05 and a correlation coefficient of 1.0.

Histamine (10 and 100 μg/kg i.d.) produced approximately 4- and 5-fold increases, respectively, in extravasation of Evans blue and 6- and 8-fold increases of fluorescein extravasation into the skin (Fig. 1). Sub-

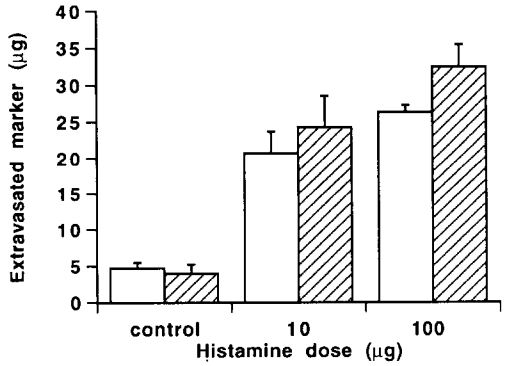

Fig. 1. Extravasation of Evans blue (open bars) and fluorescein (hatched bars) following intradermal injection of saline or histamine (10 μg/kg and 100 μg/kg) in the anaesthetised rat.

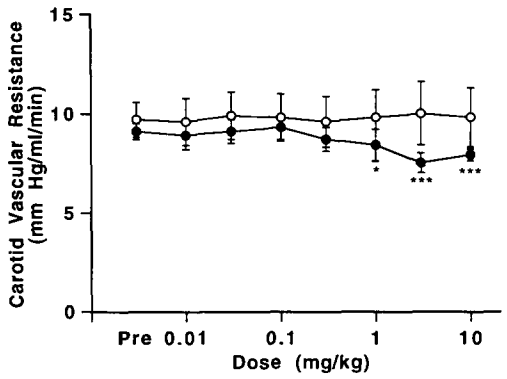

Fig. 2. Cumulative dose response curve for the effect of intravenous sumatriptan (closed circles) compared with saline control (open circles) on carotid vascular resistance in the rat. * Indicates a significant ($P < 0.05$) difference between sumatriptan and control, *** indicates $P < 0.001$)

sequent studies showed that fluorescein had increased fluorescence at pH 11 and that heating the sample was not required to release the fluorescein into solution.

3.2. Efects of sumatriptan on basal systemic and carotid haemodynamics

Baseline values for mean arterial blood pressure and carotid blood flow were 138.8 ± 4.9 mmHg and 15.6 ± 1.0 ml/min. Sumatriptan (0.01–0.3 mg/kg i.v.) had no significant effect on carotid vascular resistance, whereas doses of 1–10 mg/kg produced a significant, dose-related decrease in carotid vascular resistance compared with predose values (Fig. 2, maximal fall = 17.5 ± 2.4%, $P < 0.001$). This decrease in carotid vascular resistance was due mainly to a small fall in mean arterial blood pressure seen after high doses of sumatriptan (maximal fall = 13.8 ± 2.2 mmHg, $P < 0.001$) since sumatriptan had no effect on carotid flow except at 3 mg/kg when there was a small but statistically significant increase. A dose of 0.3 mg/kg of sumatriptan was therefore chosen for the extravasation study as this was the highest dose with no effect on mean arterial blood pressure or carotid vascular resistance.

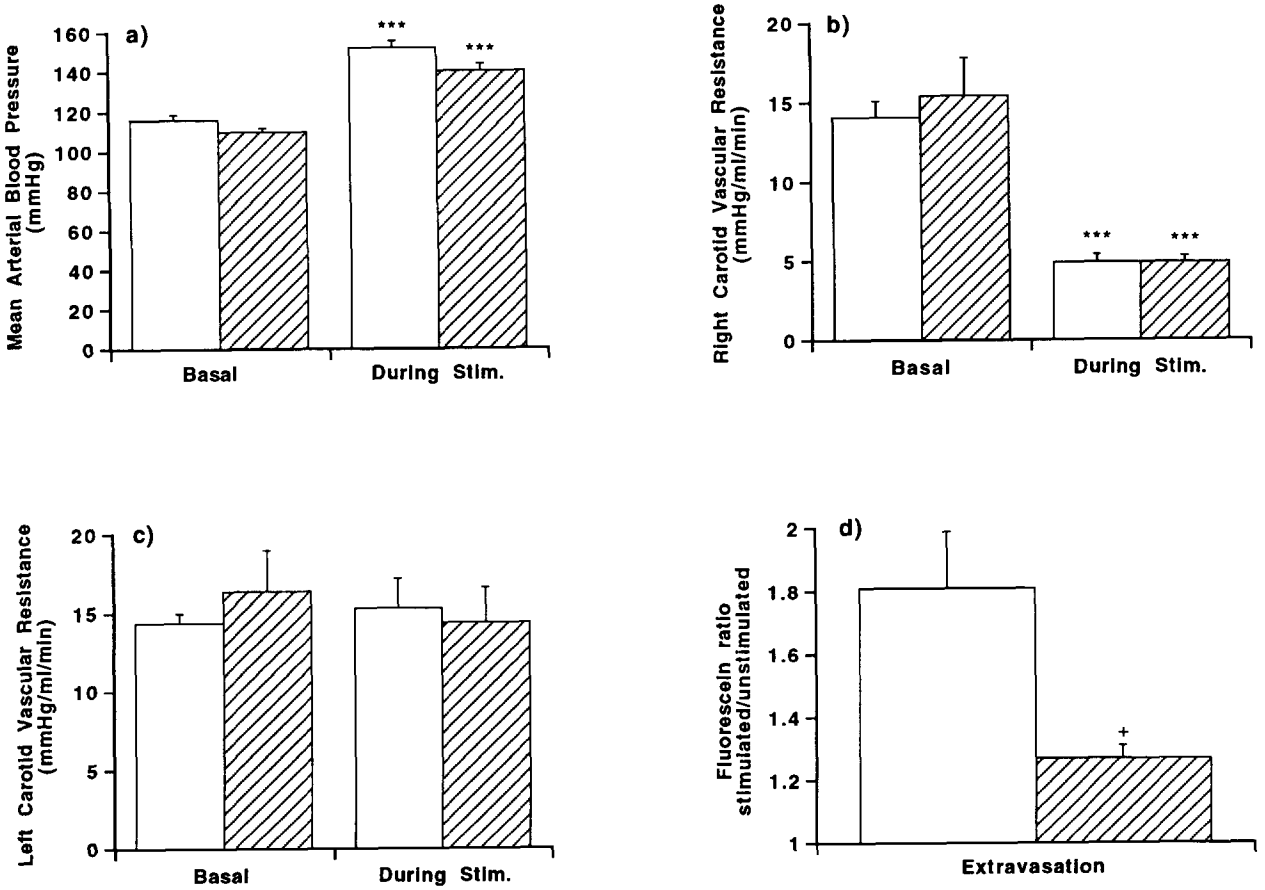

Fig. 3. The effect of sumatriptan pretreatment on the responses of (a) mean arterial blood pressure, (b) right carotid vascular resistance, (c) left carotid vascular resistance and (d) plasma protein extravasation during right trigeminal ganglion stimulation in the anaesthetised rat. Open bars indicate saline pretreatment and hatched bars indicate sumatriptan (0.3 mg/kg) pretreatment. *** Indicates a significant ($P < 0.001$) difference between basal and stimulated values while + indicates a significant ($P < 0.05$) difference between sumatriptan and control.

3.3. Trigeminal ganglion stimulation in anaesthetised rats

Basal mean arterial blood pressure was 116 ± 2.6 mmHg in control animals and 110 ± 1.8 mmHg in sumatriptan treated animals. Trigeminal ganglion stimulation produced an increase in mean arterial blood pressure of 35.1 ± 2.5 mmHg in control rats and 29.4 ± 2.4 mmHg in sumatriptan treated animals (Fig. 3a). Neither the basal blood pressure nor the increase in mean arterial blood pressure during stimulation was significantly different after sumatriptan compared with controls. Right trigeminal stimulation produced a large increase in right carotid flow from 8.5 ± 0.6 to 32.5 ± 3.7 ml/min giving a marked decrease in right carotid vascular resistance of 9.2 ± 0.7 mmHg/ml/min (Fig. 3b). It was observed that on the right side of the face both the eyelids and nose become very pink during stimulation suggesting vasodilatation. Carotid flow on the contralateral side increased slightly from 8.2 ± 0.3 to 10.6 ± 1.0 ml/min but there was no effect on left carotid vascular resistance (Fig. 3c). After sumatriptan pretreatment the carotid flow on the stimulated side increased from 8.4 ± 1.7 to 29.3 ± 2.5 ml/min and on the contralateral side from 7.9 ± 1.5 to 11.2 ± 2.1 ml/min. Thus sumatriptan had no significant effect on either the basal or stimulated carotid vascular resistance on either side (Fig. 3b,c). Trigeminal stimulation produced plasma protein extravasation into the dura mater (ratio = 1.81 ± 0.18) which was significantly reduced by pretreatment with sumatriptan (ratio = 1.27 ± 0.04, $P < 0.02$, Fig. 3d).

4. Discussion

These data demonstrate that fluorescein is a useful alternative to the ^{125}I label used as a marker of plasma protein extravasation by Moskowitz and co-workers and the control values for extravasation were very similar to those obtained by these authors. Our results also confirm that sumatriptan (0.3 mg/kg) blocks plasma protein extravasation into the dura mater following trigeminal ganglion stimulation in the anaesthetised rat (Buzzi and Moskowitz, 1990).

Trigeminal ganglion stimulation decreased ipsilateral carotid vascular resistance (presumably due to vasodilatation) but had no effect on contralateral carotid vascular resistance. Sumatriptan had no effect on either the basal or the stimulated carotid vascular resistance, neither did it have any effect on the basal mean arterial blood pressure or the stimulation induced increase in mean arterial blood pressure or in carotid blood flow. These results suggest that, at least in the rat, the inhibition by sumatriptan of plasma protein extravasation occurs independently of any change in total carotid vascular resistance. The rat appears to differ therefore from other species such as the pig, dog, cat and rabbit in which sumatriptan has been shown to selectively increase carotid vascular resistance (Den Boer et al., 1991; Feniuk et al., 1989; Perren et al., 1989; MacLennan and Martin, 1990; Middlefell and Price, 1993). In the cat Goadsby and Edvinsson (1993) showed that trigeminal ganglion stimulation increased cerebral blood flow on the stimulated side measured by laser Doppler flowmetry and that this increase was significantly attenuated by sumatriptan. In species (cat, pig) where carotid blood flow distribution was measured, the decrease in cerebral blood flow produced by sumatriptan was due to a decrease in the arterio-venous anastomotic component (Perren et al., 1989; MacLennan and Martin, 1990; Den Boer et al., 1991). It is unclear whether or not the rat possesses arterio-venous anastomoses within the craniovascular circulation. If not, or if there are relatively few compared to other species, this might account for the lack of effect of sumatriptan on carotid flow in this species.

Recently the presence of both 5-HT_{1B} and 5-HT_{1D} receptors have been demonstrated in the rat brain (Bruinvels et al., 1993a). The 5-HT_{1B} receptor is believed to be the species homologue of the human 5-$HT_{1D\beta}$ receptor although the pharmacological profiles are dissimilar. Using in situ hybridisation histochemistry in rat brain, only low densities of 5-$HT_{1D\alpha}$ mRNA were found compared to the 5-HT_{1B} mRNA, although the trigeminal ganglia seemed to have a high density of both (Bruinvels et al., 1993b). Activation of these pre-junctional trigeminal receptors by, for example sumatriptan, is believed to be responsible for the inhibition of the neurogenically mediated release of neuropeptides and concomitant plasma protein extravasation (Buzzi and Moskowitz, 1990). Trigeminal stumulation has been shown to produce increases in the level of CGRP in the cranial circulation of the cat (Goadsby and Edvinsson, 1993) and the rat superior sagital sinus (Buzzi et al., 1991). In both cases the increases in CGRP level were antagonised by sumatriptan.

Whilst 5-HT_{1B} receptors have been demonstrated to mediate vasoconstriction in rat caudal arteries (Craig and Martin, 1993) and the presence of 5-$HT_{1D\beta}$ (but not 5-$HT_{1D\alpha}$) mRNA has been demonstrated in human cerebrovascular tissue (Hamell et al., 1993), to date there is no evidence to suggest that the cranial vasculature of the rat possesses a contractile 5-HT_1-like receptor. In fact Chang and co-workers have shown that 5-HT evokes vasoconstriction in rat basilar artery via a 5-HT_2 receptor mechanism, unlike in the guinea-pig basilar artery which is 5-HT_1-like (Chang et al., 1988, Chang and Owman, 1989)

It is also possible however that sumatriptan does produce vasoconstriction in specific vessels within the

cranial vasculature of the rat but that the proportion of sumatriptan-sensitive vessels in the total carotid vasculature of the rat may be too small to have any measurable effect on overall carotid vascular resistance. In the pig it has been shown that while systemic administration of sumatriptan does indeed reduce overall arterio-venous anastomotic flow sumatriptan had no effect on cerebral blood flow or on blood flow in the dura mater (Den Boer et al., 1992a,b).

It has been shown using a pial window technique that local application of sumatriptan onto rat pial arteries and arterioles produces a dose-dependent constriction whereas intravenous infusion of sumatriptan has no effect on cerebral blood flow measured using radioactive microspheres (Pryke, 1992). This suggests that systemically administered sumatriptan could have local effects on pial (and dural) vessel diameter without measurable effect on the total carotid flow. However, in the cat sumatriptan caused a decrease in pial artery diameter when injected perivascularly but no effect on pial artery diameter when injected systemically although it did cause selective carotid vasoconstriction (Connor et al., 1992). These results suggest that sumatriptan does not readily cross the blood-brain barrier. Indeed, it has been shown that sumatriptan can decrease the peak-to-peak amplitude of trigeminal evoked potentials only after the blood-brain barrier has been disrupted by infusion of a hyperosmotic mannitol solution (Kaube et al., 1993). It is not known whether the blood-brain barrier is disrupted during a migraine attack. It has been shown that sumatriptan does not alter the middle cerebral artery diameter outside of a migraine attack but during a migraine attack the middle cerebral artery is dilated on the headache side only and this is normalised by sumatriptan (Friberg et al., 1991). It would be of great interest to know if the blood-brain barrier is indeed disrupted during a migraine attack (and/or trigeminal stimulation) as this may help considerably in elucidating the site and mechanism of action of antimigraine drugs.

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