ELSEVIER

European Journal of Pharmacology 347 (1998) 71–75

ejp

Short communication

Depressor responses to endothelin-1 into the superior colliculus of rats: predominant role of endothelin ET_B receptors

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Received 6 March 1998; accepted 10 March 1998

Abstract

We used in vitro autoradiography to identify the endothelin-1 receptor subtype(s) in the superficial gray layer of the superior colliculus of rats. These studies showed dense binding of (3-[^{125}I]iodotyrosyl^{13})-[Ala^{11,15}]Ac-endothelin-1-(6-21) (BQ3020) (for endothelin ET_B receptors), while tissues incubated with [^{125}I](*N*-(hexahydro-1-azepinyl)carbonyl)L-Leu(1-Me)D-Trp-D-Tyr (PD151242) (for endothelin ET_A receptors) had low binding. In addition, we examined the effects of the endothelin receptor antagonists, (*R*)-2-[(*R*)-2-[(*S*)-2-[[1-hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]-amino-3-(2-pyridyl)propionic acid (FR139317) (endothelin ET_A receptor-selective), (+)-(1*S*,2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid (SB209670) (endothelin ET_A/ET_B receptor non-selective) and *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-metLeu-D-1-methoxy-carbonylTrp-D-Nle (BQ-788) (endothelin ET_B receptor-selective antagonist) on the responses following administration of endothelin-1 into the superficial layer of the superior colliculus. Endothelin-1 microinjected into this nucleus causes decreases in blood pressure (control, 109 ± 6 mmHg, $n = 6$; endothelin-1, 72 ± 5 mmHg, $n = 6$). These effects were greatly reduced by pre-administration into the superior colliculus of BQ-788 (5 nmol/rat) or SB209670 (3 nmol/rat) (94 ± 5 and 98 ± 6%, respectively) but were not affected by FR139317 (5 nmol/rat) (6 ± 3%). Therefore, together with autoradiography, our functional data showed that endothelin ET_B receptors are the predominant mediators of depressor responses induced by endothelin-1 injected into the superior colliculus of the rat. © 1998 Elsevier Science B.V.

Keywords: Endothelin-1; Superior colliculus; Endothelin ET_B receptor; Blood pressure

1. Introduction

Autoradiographic studies have demonstrated that endothelin-1 binds selectively throughout the midbrain of rats, and notably in the superior colliculus (Kohzuki et al., 1991). This is interesting, for the superior colliculus, in addition to integrating visual inputs, takes part in the central control of cardiovascular function via descending neuronal pathways originating in its superficial, intermediate or deep layer (Keay and Dean, 1988). Recently, we showed that endothelin-1 microinjected into the superficial layer of superior colliculus induces decreases in blood pressure (D'Amico et al., 1996). However, no study has been done in which endothelin receptors mediate the response to endothelin-1 injected into the superior colliculus. Therefore, we have used in vitro autoradiographic studies to identify endothelin-1 receptor subtypes within the superior colliculus. We have also examined the effects of the endothelin receptor antagonists, (*R*)-2-[(*R*)-2-[(*S*)-2-[[1-hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]-amino-3-(2-pyridyl)propionic acid (FR139317) (endothelin ET_A receptor-selective) (Sogabe et al., 1992), (+)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid (SB209670) (endothelin ET_A/ET_B receptor non-selective) (Ohlstein et al., 1989) and *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-metLeu-D-1-methoxy-carbonylTrp-D-Nle (BQ-788) (endothelin ET_B receptor-selective antagonist) (Ishikawa et al., 1994) on the responses following injection administration of endothelin-1 into the superficial layer of the superior colliculus.

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Endothelin-1 was used at a single dose of 10 pmol which certainly produces a depressor response (D'Amico et al., 1996).

2. Materials and methods

2.1. Autoradiographic study

Autoradiography was performed using four rats anaesthetized with sodium pentobarbitone (60 mg/kg i.p.) and then perfused intracardially with ice-cold 100 mM phosphate buffer (pH 7.4) containing 300 mM sucrose and lightly fixed with 0.1% formaldehyde in phosphate/sucrose buffer. The brains were removed and 10 μm sections were cut in a cryostat at -20°C and thaw-mounted on to gelatinized microscope slides. Endogenous peptide levels were reduced by preincubating slide-mounted tissue in 50 mM tris buffer. [3-[^{125}I]iodotyrosyl^{13}]-endothelin binding was determined in the presence of 150 pM of radioligand (Dashwood et al., 1994) and receptor subtypes were identified using [^{125}I](*N*-(hexahydro-1-azepinyl)carbonyl)L-Leu(1-Me)D-Trp-D-Tyr ([^{125}I]PD151242) (endothelin ET_A receptors, Davenport et al., 1994) and (3-[^{125}I]iodotyrosyl^{13})-[Ala^{11,15}]Ac-ET-1($_{6\text{-}21}$) ([^{125}I]BQ3020) (endothelin ET_B receptors, Molenaar and Davenport, 1992). Non-specific binding was established by incubating sections in the presence of 1 μM endothelin-1. Autoradiographs were generated by exposing sections to Hyperfilm ^{3}H (Amersham International, Amersham, UK) as described previously (Moody et al., 1990). Tissue sections were stained with haematoxylin and eosin or neutral red for subsequent histology. Autoradiographs and tissue sections were then viewed on a Nikon macro system and photographed when appropriate.

2.2. In vivo experiments

Male Wistar rats (190–230 g) were anaesthetised with urethane (1.2 g/kg, i.p.) and the right femoral artery was cannulated for the measurement of blood pressure (pressure transducer, Elcomatic type 750). The animals, while spontaneously breathing, were then placed in a stereotaxic head frame and the dorsal surface of the brain was exposed by craniotomy to permit intra-superior colliculus microinjections using a Hamilton 1-μl syringe supported in a stereotaxic micromanipulator. The coordinates of the atlas of Paxinos and Watson (1986) (measured in mm from the bregma: posterior, -6.30; lateral, 0.1; vertical, 3.2) were used to position the needle tip of the microsyringe. Another catheter was inserted into a jugular vein for the administration of saline (1.5 ml/h) to compensate for any fluid loss. Under these conditions, rectal temperature was monitored and maintained between 37 and 38°C by means of a probe linked to a homeothermic blanket (Bioscience, Sheerness, UK). Intra-SC injections were given in a total volume of 100 nl over of a period of 5 s. After the experiments, the positioning of the injection site was checked histologically. For this, 100 nl methylene blue (0.2%) was injected intracerebrally 5 min before the rat was killed with a high dose of sodium pentobarbitone (200 mg/kg, i.v.). Each animal was then perfused intracardially with 50 ml of phosphate-buffered saline followed by 50 ml of a 10% formalin solution in phosphate-buffered saline. The brain was removed and immersed in saturated formalin for 24 h. The injection site was verified using two consecutive sections (40 μm), one stained with Cresyl violet to identify nuclei and the other unstained, to determine dye diffusion.

2.2.1. Experimental protocol

After a 30-min stabilisation period endothelin-1 (10 pmol) was injected into the superficial layer of the superior colliculus as consecutive injections at dose of 10 pmol, each injection being made when the blood pressure had returned to its basal value, in order to investigate the reproducibility of the depressor response to endothelin-1. After this, antagonist studies were performed in a separate series of experiments with endothelin-1 and two different doses of each antagonist (5 and 50 nmol for FR139317; 0.5 and 5 for BQ-788; or 0.3 and 3 nmol for SB209670) administered as cumulative doses to the same animal. In these studies, initially the lowest dose of antagonist was microinjected, followed 10 min later by injection of endothelin-1. If the response to endothelin was unaffected, the highest dose of antagonist was given followed by another injection of endothelin-1. Each antagonist was tested in separate groups of animals. Arterial blood pressure was continuously monitored throughout. Heart rate was derived from lead II electrocardiograms recorded from subdermal platinum electrodes on a Hellige cardiotest EK41 (Hellige, Freiburg, Germany). The effects on blood pressure and heart rate of treatment of the superior colliculus with endothelin antagonists alone were also examined.

2.3. Materials

Endothelin-1 was purchased from the Peptide Institute (Osaka, Japan), and urethane from Sigma (Poole, UK). Endothelin-1 was reconstituted in 0.1% v/v acetic acid and then dissolved in 0.9% w/v saline containing 0.1% w/v bovine serum albumin and 10 mM sodium bicarbonate. [[^{125}I]endothelin-1 (specific activity 2000 Ci/mmol), [[^{125}I]]PD151242, [[^{125}I]]BQ3020, Hyperfilm ^{3}H were all purchased from Amersham International. For the in vivo experiments, FR139317 (*R*)-2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indoly)]propionyl]-amino-3-(2-

pyrydil)propionic acid) (Parke Davis Pharmaceutical Research) (an endothelin ET_A receptor-selective antagonist), SB209670 (+)-(1*S*,2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid) (Banyu Pharmaceutical, Japan) (an endothelin ET_A/ET_B receptor non-selective antagonist) and BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-metLeu-*d*-L-methoxy-carbonylTrp-D-Nle) (Hoechst, Frankfurt, Germany) (an endothelin ET_B receptor-selective antagonist) were dissolved in 0.9% NaCl. Control injections were carried out with saline (0.9% NaCl) containing the same amount of solvent as the drug solutions. These did not produce any changes in blood pressure.

2.4. Statistics

All results are expressed as means ± S.E.M., with $P < 0.05$ being considered significant. Changes in blood pressure or heart rate were compared by analysis of variance (ANOVA) and Newman–Keuls test for multiple comparisons (Tallarida and Murray, 1987).

3. Results

3.1. Autoradiographic study

There was a dense binding of [^{125}I]endothelin-1 to various areas of the rat brain, as described previously (Kohzuki et al., 1991). Binding was reduced by > 90% when sections were incubated in the presence of excess unlabelled peptide (non-specific binding < 10%) as assessed in a gamma counter. Binding to endothelin ET_B sites was associated with the hippocampus and regions such as the cortex and the superior colliculus with most of the binding being within the superficial layer (Fig. 1A). Binding of [^{125}I]PD151242 (endothelin ET_A sites) was predominately confined to the hippocampus, amygdala and the periaqueductal gray area (Fig. 1C).

3.2. Effects of local injection of endothelin $ET_{A/B}$ receptor antagonists on the effects on blood pressure or heart rate induced by endothelin-1 into the superior colliculus

The basal mean blood pressure of the rats was 109 ± 6 mmHg ($n = 6$). This was decreased (32 ± 3%) by endothe-

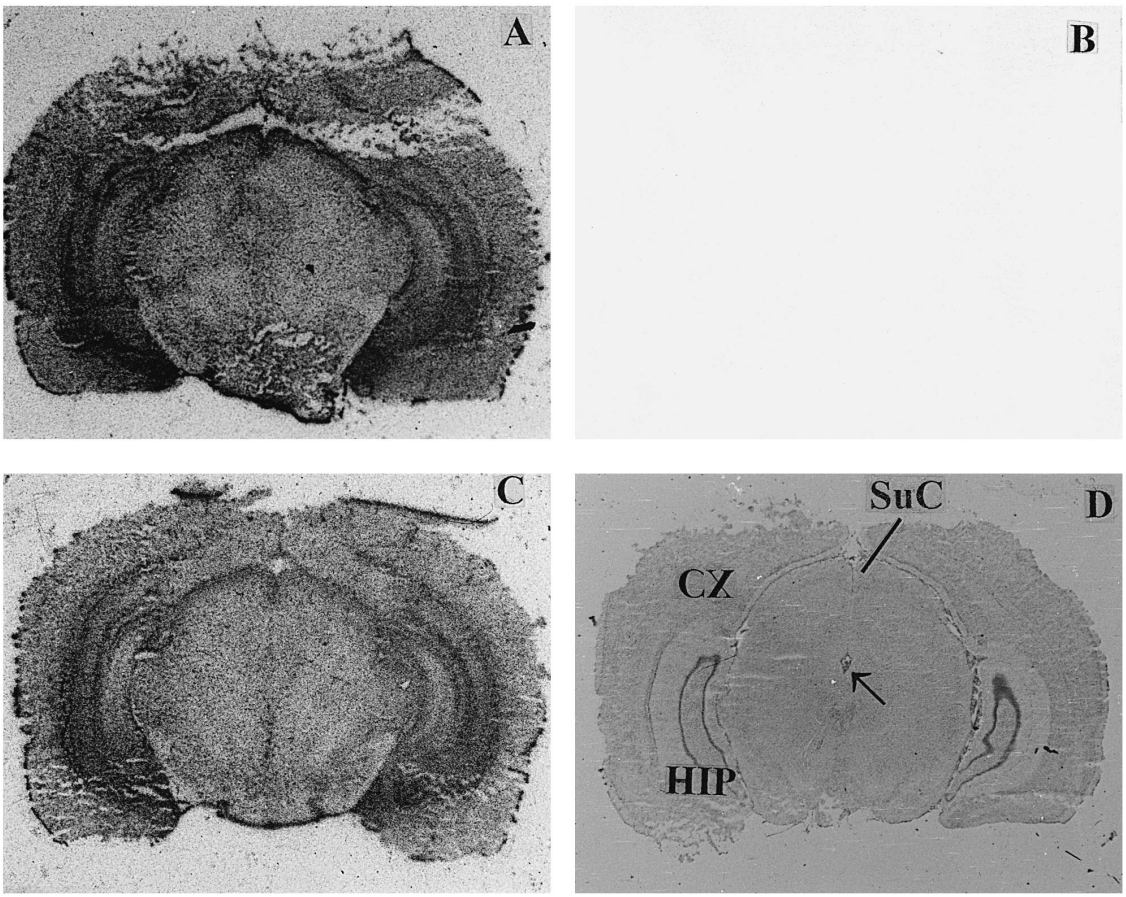

Fig. 1. Autoradiographic localization of ET-1 binding in rat brain. (A) Film autoradiograph of [^{125}I]BQ3020 (ET_B sites) binding to coronal section of rat brain. (B) Binding to section incubated with [^{125}I]BQ3020 in the presence of 1 μM unlabelled ET-1 (non-specific binding). (C) Autoradiograph from brain section incubated with [^{125}I]PD151242 (ET_A sites). (D) Representative brain section (mm from the bregma: posterior, −6.30; lateral, 0.1; vertical, 3.2; level according to atlas of Paxinos and Watson, 1986) stained with neutral red. Arrow indicates aqueduct, SuC = superficial gray layer of the superior colliculus, cx = cortex, hip = hippocampus. Scale bar = 250 μm.

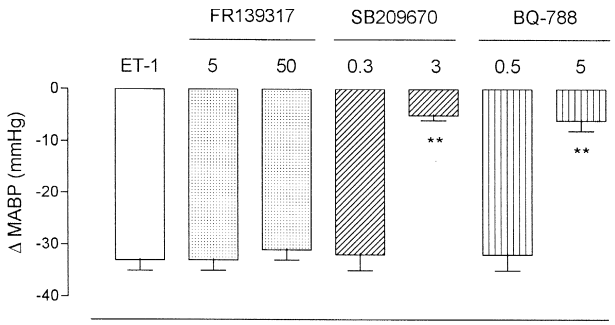

Fig. 2. Changes in mean arterial blood pressure (ΔMABP) (mmHg ± S.E.) after microinjection of ET-1 (10 pmol) into the superficial layer of the superior colliculus (SC) of rats treated 10 min before with FR139317, BQ-788, SB209670 or vehicle. Each column represents the mean of six observations = S.E. Significant differences from groups treated with ET-1 are shown as $** P < 0.01$.

lin-1 (10 pmol) microinjected into the superficial layer of the superior colliculus (Fig. 2). Endothelin-1 did not affect the heart rate (endothelin-1, 394 ± 6 beats/min, $n = 6$; control, 401 ± 7 beats/min, $n = 6$). The decreases in blood pressure induced by endothelin-1 were greatly reduced by pre-administration to the superior colliculus of BQ-788 (5 nmol/rat) or SB209670 (3 nmol/rat) (94 ± 5 and 98 ± 6%, $P < 0.01$, $n = 6$, respectively), but not affected by FR139317 (5 nmol/rat) (6 ± 3%, $P > 0.05$, $n = 6$). These had no effects on mean arterial blood pressure when injected alone into the superior colliculus. Similarly, the antagonists did not influence heart rate when they were injected into the superior colliculus prior to endothelin-1 (control, 398 ± 7; BQ-788, 402 ± 9; SB209670, 400 ± 7; FR139317, 399 ± 10). BQ-788 (0.5 nmol), SB209670 (0.3 nmol) or FR139317 (50 nmol) had no effects on endothelin-induced changes in arterial blood pressure ($n = 4$) (Fig. 2).

4. Discussion

In vitro autoradiography revealed endothelin ET_B receptor binding in the superficial layer of the superior colliculus, as indicated by [^{125}I]BQ3020. Binding of [^{125}I]PD151242 was low, indicating little involvement of endothelin ET_A receptors into this nucleus. Together with autoradiography, our data from functional experiments using selective and non-selective antagonists, allowed us to identify the receptor(s) mediating the endothelin-1-induced effects discussed above. The facts that the endothelin ET_B receptor antagonist, BQ-788, or the endothelin ET_A/ET_B non-selective receptor antagonist, SB209670, reduced the changes induced by endothelin-1 injected into the superior colliculus, leads to the suggestion that the changes thus induced are principally mediated by endothelin ET_B receptors. This is in spite of the fact that both endothelin ET_A and ET_B receptors can mediate vasoconstriction following systemic administration of endothelin-1 (Warner et al., 1993; Fukuroda et al., 1994). Endothelin ET_A receptors are little involved in this response. Thus, endothelin ET_B receptors are most probably the predominant receptor type mediating the actions of exogenous endothelin-1 in the superior colliculus of the rat. However, the failure of endothelin receptor antagonists to affect blood pressure themselves raises the question as to whether endogenously produced endothelin-1 does act within the superior colliculus to control blood pressure. This question would require further investigation.

Acknowledgements

This study was supported by National Council of Research, Rome, Italy.

References

D'Amico, M., Rossi, F., Warner, T.D., 1996. Cardiovascular responses induced by injection of endothelin-1 into the superior colliculus of rats. Eur. J. Pharmacol. 316, 245–247.

Dashwood, M.R., Allen, S.P., Luu, T.N., Muddle, J.R., 1994. The effect of the ET_A receptor antagonist, FR139317, on [^{125}I]-ET-1 binding to the atherosclerotic human coronary artery. Br. J. Pharmacol. 112, 386–389.

Davenport, A.P., Kuc, R.E., Fitzgerald, F., Maguire, J.J., Berryman, K., Doherty, A.M., 1994. [^{125}I]PD151242: a selective radioligand for human ET_A receptors. Br. J. Pharmacol. 111, 4–6.

Fukuroda, T., Ozaki, S., Ihara, K., Ishikawa, K., Yano, M., Nishikibe, M., 1994. Synergistic inhibition by BQ-123 and BQ-788 of endothelin-1-induced contractions of the rabbit pulmonary artery. Br. J. Pharmacol. 113, 336–338.

Ishikawa, K., Ihara, M., Noguchi, K., Mase, T., Mino, N., Saeki, T., Fukuroda, T., Fukami, T., Ozaki, S., Nagase, T., Nishikibe, M., Yano, M., 1994. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. Proc. Natl. Acad. Sci. USA 91, 4892–4896.

Keay, K.A., Redgrave, P., Dean, P., 1988. Cardiovascular and respiratory changes elicited by stimulation of rat superior colliculus. Brain Res. Bull. 20, 13–17.

Kohzuki, M., Chay, S.H., Paxinos, G., Karavas, A., Casley, D.J., Johnston, C.J., Mendelshon, F.A., 1991. Localization and characterization of endothelin receptor binding sites in the rat brain visualized by in vitro autoradiography. Neuroscience 42, 245–260.

Molenaar, P., Kuc, R.E., Davenport, A.P., 1992. Characterization of two new ET_B selective radioligands, [^{125}I]BQ3020 and [^{125}I]-[Ala^{1,3,11,15}]-ET-1 in human heart. Br. J. Pharmacol. 107, 637–639.

Moody, C.J., Dashwood, M.R., Sykes, R.M., Chester, M., Jones, S.M., Yacoub, M.H., Harding, S.E., 1990. Functional and autoradiographic evidence for endothelin-1 receptors on human and rat myocytes. Circ. Res. 67, 764–769.

Ohlstein, E.H., Nambi, P., Douglas, S.A., Edwards, R.M., Gellai, M., Lago, A., Leber, J.D., Cousins, R.D., Gao, A., Frazee, J.S., Peishoff, C., Bean, J.W., Eggleston, D.S., Elshourbagy, N.A., Kumar, C., Lee, J.A., Yue, T., Louden, C., Brooks, D.P., Weinstock, J., Feuerstein, G., Poste, G., Ruffolo, R., Gleason, J.G., Elliot, J.D., 1989. SB209670,

a rationally designed potent nonpeptide endothelin receptor antagonist. Proc. Natl. Acad. Sci. USA 91, 8052–8056.

Paxinos, G., Watson C., 1986. The Rat Brain in the Stereotaxic Coordinates. Academic Press, San Diego.

Sogabe, K., Nirei, H., Shoubo, M., Hamada, K., Nomoto, A., Nemi, K., Notsu, Y., Ono, T., 1992. A novel endothelial receptor antagonist: studies with FR139317. J. Vasc. Res. 29, 201–202.

Tallarida, R.J., Murray R.B., 1987. Manual of Pharmacologic calculations with Computer Programs. Springer-Verlag, New York.

Warner, T.D., Allcock, G.H., Corder, R., Vane, J.R., 1993. Use of the endothelin antagonists BQ-123 and PD142893 to reveal three endothelin receptors mediating smooth muscle contraction and the release of EDRF. Br. J. Pharmacol. 110, 777–782.