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Autoradiographic distribution of adrenomedullin receptors in the rat brain

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Abstract

The autoradiographic distribution of putative brain adrenomedullin receptors was investigated using [^{125}I]human adrenomedullin$_{13-52}$ as a new radioligand. Specific [^{125}I]human adrenomedullin$_{13-52}$ binding sites were very discretely distributed in the rat brain with enrichment seen in the choroid plexus and linings of the third, fourth and lateral ventricles, basolateral amygdaloid nuclei, neural lobe of the pituitary gland, the trigeminal nerves and in the granular cell layer of the cerebellum. To our knowledge, this is the first report on the discrete localization of adrenomedullin receptors in the mammalian brain. © 2001 Published by Elsevier Science B.V.

Keywords: Adrenomedullin; Receptor distribution; [^{125}I]human adrenomedullin$_{13-52}$ radioligand

Adrenomedullin is a 52-amino acid peptide acting as a potent vasodilator (Kitamura et al., 1993). Peripheral or intracerebroventricular injections of adrenomedullin induced biological effects that are similar but not identical to those of calcitonin gene-related peptides (CGRP) (for recent reviews, see Jacques et al., 2000; Juaneda et al., 2000a). These results suggested the existence of distinct adrenomedullin and CGRP receptors in the central nervous system.

Receptor binding assays have revealed the existence of high affinity specific [^{125}I]human adrenomedullin binding sites in brain membrane preparations (Sone et al., 1997). However, using this radioligand, it was not possible to obtain adequate autoradiographic data on the discrete distribution of adrenomedullin receptors due to the very high levels of nonspecific binding generated by this radioligand (unpublished observations). Accordingly, the development of better probes to study brain adrenomedullin receptor binding sites was deemed to be a priority. Various fragments were considered and we recently focussed on [^{125}I]human adrenomedullin$_{13-52}$ because of its much lower level of nonspecific labeling (Juaneda et al., 2000b). We have investigated here the distribution of adrenomedullin receptors in the rat brain using [^{125}I]human adrenomedullin$_{13-52}$ as a new radioligand.

Male Sprague–Dawley rats (250 g) obtained from Charles River Canada (St. Constant, Quebec, Canada) were kept on a 12-h light/dark cycle (light on at 7:00 a.m.) in temperature- and humidity-controlled rooms. Animals were fed with standard laboratory chow and had accessed to tap water ad libitum. Animal care was provided according to protocols and guidelines approved by McGill University and the Canadian Council of Animal Care. Rats were decapitated and their brains rapidly removed from the skull, frozen in 2-methylbutane at −40°C and then kept at −80°C until used. Serial coronal sections (20 μm) were cut and thaw-mounted onto polylysine-coated slides, dried overnight in a desiccator at 4°C, and then kept at −80°C.

Receptor autoradiography of [^{125}I]human adrenomedullin$_{13-52}$ binding sites in the rat brain was performed by preincubating adjacent coronal sections for 60 min at room temperature in 50 mM Tris–HCl buffer, 100 mM NaCl and 4 mM $MgCl_2$, pH 7.4, followed by a 120-min incubation in fresh buffer containing 35 pM [^{125}I]human adrenomedullin$_{13-52}$ (New England Nuclear, Boston, MA, USA), 0.2% bovine serum albumin (Sigma, St Louis, MO, USA), 0.4 mM bacitracin (Sigma), 4 μg/ml leupeptin (Sigma) and 2 μg/ml chymostatin (Sigma). Sections were then washed in ice-cold buffer four times (2 min each) and

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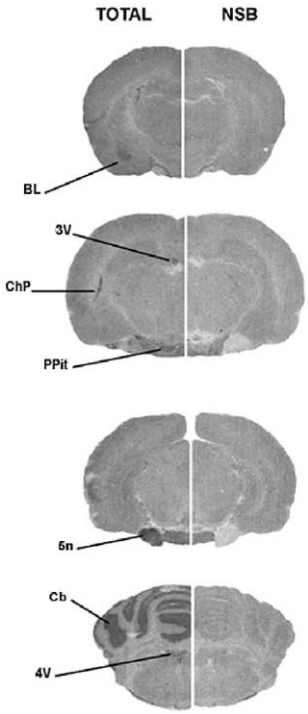

Fig. 1. Photomicrographs of the autoradiographic distribution of [^{125}I]human adrenomedullin$_{13-52}$ binding sites in the rat brain. TOTAL represents total binding while NSB represents nonspecific binding remaining in the presence of 1 μM human adrenomedullin$_{13-52}$. Abbreviations: 3V and 4V; third and fourth ventricles; 5n, trigeminal nerve; BL, basolateral amygdaloid nuclei; Cb, cerebellum; ChP, choroid plexus; PPit, neuronal lobe of the pituitary.

dipped twice in cold distilled water. Nonspecific binding was determined in the presence of 1 μM human adrenomedullin$_{13-52}$ (Phoenix Pharmaceuticals, Mountain View, CA, USA). Incubated sections were exposed to tritium sensitive films ([^{3}H]-Hyperfilm, Amersham Canada, Ontario, Canada) for 6 days.

The very discrete distribution of specific [^{125}I]human adrenomedullin$_{13-52}$ binding sites in rat brain is shown in Fig. 1. Specific [^{125}I]human adrenomedullin$_{13-52}$ binding sites are located in the choroid plexus and linings of the third, fourth and lateral ventricles, the basolateral amygdaloid nuclei, the neural lobe of the pituitary gland, the trigeminal nerves and the granular cell layer of the cerebellum. No or very low levels of specific [^{125}I]human adrenomedullin$_{13-52}$ binding sites were detected in other brain regions (Fig. 1).

The present study reports for the first time on the discrete distribution of specific, high affinity [^{125}I]human adrenomedullin$_{13-52}$/adrenomedullin binding sites in selected areas of the rat brain. The observed localization of specific [^{125}I]human adrenomedullin$_{13-52}$/adrenomedullin binding sites in the rat brain is much more restricted than that of specific [^{125}I]human CGRPα/CGRP receptors (Jacques et al., 2000; Van Rossum et al., 1995) and is rather distinct from those observed for possible related receptor mRNAs including CRLR mRNA (a CGRP or an adrenomedullin receptor phenotype depending on the associated RAMP), RDC-1 mRNA or rat L1 mRNA receptor distribution (Oliver et al., 1998). However, some overlaps also exist, for example in the basolateral nucleus of the amygdala, the choroid plexus and the linings of the ventricles (Oliver et al., 1998). It thus appears that [^{125}I]human adrenomedullin$_{13-52}$ recognizes a class of very discretely distributed adrenomedullin receptor sites in the rat brain that are clearly distinct from CGRP receptors. Studies are in progress to establish the relationship between these specific [^{125}I]human adrenomedullin$_{13-52}$ binding sites, CRLR, RDC-1 and the RAMPs.

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