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Quantitative receptor radioautography in the study of receptor-receptor interactions in the nucleus tractus solitarii

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Abstract

The nucleus tractus solitarii (NTS) in the dorsomedial medulla comprises a wide range of neuropeptides and biogenic amines. Several of them are related to mechanisms of central blood pressure control. Angiotensin II (Ang II), neuropeptide Y (NPY) and noradrenaline (NA) are found in the NTS cells, as well as their receptors. Based on this observation we have evaluated the modulatory effect of these peptide receptors on α_2-adrenoceptors in the NTS. Using quantitative receptor radioautography, we observed that NPY and Ang II receptors decreased the affinity of α_2-adrenoceptors for their agonists in the NTS of the rat. Cardiovascular experiments agreed with the *in vitro* data. Coinjection of a threshold dose of Ang II or of the NPY agonists together with an ED_{50} dose of adrenergic agonists such as NA, adrenaline and clonidine counteracted the depressor effect produced by the α_2-agonist in the NTS. The results provide evidence for the existence of an antagonistic interaction between Ang II AT1 receptors and NPY receptor subtypes with the α_2-adrenoceptors in the NTS. This receptor interaction may reduce the transduction over the α_2-adrenoceptors which can be important in central cardiovascular regulation and in the development of hypertension.

Key words
- Quantitative radioautography
- Nucleus tractus solitarii
- Alpha-2-adrenoceptors
- Neuropeptide Y receptors
- Angiotensin II receptors

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The nucleus tractus solitarii (NTS) in the medulla oblongata has a crucial role in central cardiovascular control. This nucleus receives primary afferents which derive from visceral receptors such as cardiopulmonary, gut, hepatic and pancreatic receptors, chemoreceptors, and baroreceptors via the vagus and glossopharyngeal nerves (1,2). Several lines of evidence suggest that the NTS, particularly its medial portion, is absolutely essential to baroreceptor reflex integrity (1,3). Lesions or pharmacological blockade of the NTS effectively eliminate the baroreceptor reflex responses (3). Electrical or pharmacological activation of the medial NTS mimics baroreceptor reflex responses, i.e., causes a reduction in mean arterial blood pressure, heart rate and sympathetic nerve activity similar to that induced by electrical stimulation of the aortic depressor nerve, the carotid sinus nerve or the nerve trunk containing baroreceptor afferents (1,3). Furthermore, the NTS is a converging area for the information from other cardiovascular centers. Electrophysiological techniques have confirmed that afferent fibers from other brainstem and higher brain nuclei may exert a modulatory role in the activity of the NTS neurons induced by activation of peripheral cardiovascular afferent fibers. The NTS neurons activated by stimulation of baroreceptor and visceral afferent nerves can also respond to electrical stimulation of the cerebellum (4), the parabrachial nucleus (5), the hypothalamus (2), the ventrolateral medulla (6) and the central nucleus of the amygdaloid complex (7). Thus, the NTS acts as an important cardiovascular center by integrating the cardiovascular information from peripheral receptors and other cardiovascular centers.

Another outstanding feature of the NTS is the large quantity and diversity of neurotransmitters/neuromodulators and their receptors localized in this nucleus (1,8). Non-peptidergic systems such as biogenic amines (9,10), histamine (11) and acetylcholine are present (12), as well as most of the known mammalian peptidergic systems including thyrotropin-releasing hormone (13), corticotropin-releasing factor (14), vasopressin (15), oxytocin (16), adrenocorticotropic hormone (17), ß-endorphin (18), dynorphin (19), enkephalins (20), substance P (21), vasoactive intestinal polypeptide (22), bombesin (23), galanin (24), neurotensin (25), calcitonin gene-related peptide (26), angiotensin II (27), and neuropeptide Y (28).

Among these neurotransmitters, the catecholamines, neuropeptide Y (NPY) and angiotensin II (Ang II) are of special interest concerning central cardiovascular control (29-31). Evidence suggests a central inhibitory role of catecholamines in the regulation of blood pressure and heart rate since decreases in blood pressure and heart rate were observed following intraventricular injection of noradrenaline into the NTS (32). NPY in the NTS possesses cardiovascular effects similar to those observed for catecholamines, leading to a prolonged decrease in arterial pressure and heart rate (33,34). Ang II is also well characterized as a participant in the mechanisms of blood pressure control (29,31). Ang II injected into the brain ventricular system leads to an increase in blood pressure. In the NTS Ang II seems to have a more variable effect, i.e., a depressor action at low doses followed by a pressor response (31). It is also suggested that Ang II inhibits the baroreceptor reflex (35).

These neurotransmitters share another interesting feature. NPY and Ang II coexist in catecholaminergic cell bodies and nerve terminals (36,37). Furthermore, an overlap of the α_2-adrenoceptor (38,39), NPY receptor (40) and Ang II receptor (41,42) distribution is observed in the NTS (Figure 1).

These morphological substrates suggest that these neurotransmission lines may interact with one another in the NTS. In support of this view, it has been shown that NPY and adrenaline injected intraventricularly in the awake rat can antagonize the hypotensive actions of one another (43). It has also been shown that NPY reduces the affinity of [^{3}H]-p-aminoclonidine ([^{3}H]-PAC) binding sites in membrane preparations from the rat dorsomedial medulla (30).

These findings and the existence of interaction among other neurotransmission lines in different brain areas such as between substance P and 5-HT receptors (44), cholecystokinin and dopamine D2 receptors (45), neurotensin and dopamine D2 receptors (46) led us to evaluate carefully the possible existence of an interaction among the neu-

Table 1 - Summary of the results obtained by the modulation of the α_2-adrenoceptors by NPY and Ang II receptors.

The binding parameters were obtained from saturation curves of [^{3}H]-PAC in the NTS using quantitative receptor radioautography. Cardiovascular parameters were obtained by injection of α_2-adrenoceptor agonists into the NTS and simultaneous recording of blood pressure in anesthetized animals.

	Binding parameters		Cardiovascular parameters	
	K_D	B_{max}	Hypotension	Bradycardia
NPY	↑	unchanged	counteracted	counteracted
Ang II	↑	↑	counteracted	unchanged

rotransmission lines involved in cardiovascular control in the NTS.

The experiments were designed to evaluate *in vitro* the alterations in binding parameters of one specific receptor in the presence of another receptor system being activated at the same time. Furthermore, *in vivo* experiments were performed in order to obtain insights about the physiological meaning of those interactions.

Table 1 summarizes the results of the analysis of the modulation of the α_2-adrenoceptors by peptide receptors such as NPY and Ang II.

The *in vitro* modulation of the α_2-adrenoceptors was evaluated by analyzing the binding parameters of [^{3}H]-PAC, an α_2-adrenoceptor agonist, in the presence or absence of different concentrations of NPY (40) and of Ang II (38). We employed quantitative radioautography which provides a good anatomical resolution for studying the NTS and is also a sensitive method for determining binding parameters.

The K_D (dissociation constant) value of [^{3}H]-PAC was increased in the presence of NPY and Ang II. This means that the affinity of the α_2-adrenoceptor for [^{3}H]-PAC was reduced in the presence of NPY and Ang II.

Noradrenaline and other α_2-adrenoceptor agonists such as adrenaline and clonidine (47,48) induce a decrease in blood pressure when injected into the NTS. The modulation of α_2-adrenoceptors *in vivo* was evaluated by injecting into the NTS an ED_{50} dose of an α_2-adrenoceptor agonist such as noradrenaline in the presence or absence of a threshold dose of NPY or Ang II. A threshold dose was chosen as a dose that had no effect on blood pressure or heart rate. We observed that NPY (40) and Ang II (38) counteracted the decrease in blood pressure triggered by the injection of noradrenaline into the NTS.

The *in vivo* findings are in line with the data obtained with quantitative radioautography. *In vitro*, NPY and Ang II decreased the affinity of the α_2-adrenoceptor for its agonist which could lead to a reduced transduction over the activated α_2-adrenoceptor in the NTS.

These modulatory effects of peptides on the α_2-adrenoceptor seem to be specific for areas rich in Ang II receptors and α_2-adrenoceptors like the NTS. No modulatory effect by Ang II was observed on the α_2-adrenoceptor in the amygdala (38), an area exhibiting a high density of α_2-adrenoceptors (49) but very low levels of Ang II receptors (50).

In support of these findings, an antagonistic interaction between Ang II AT1 receptors and α_2-adrenoceptors in the ventrolateral medulla has been described since angiotensin III was able to counteract the vasodepressor and bradycardic effects of guanabenz (51).

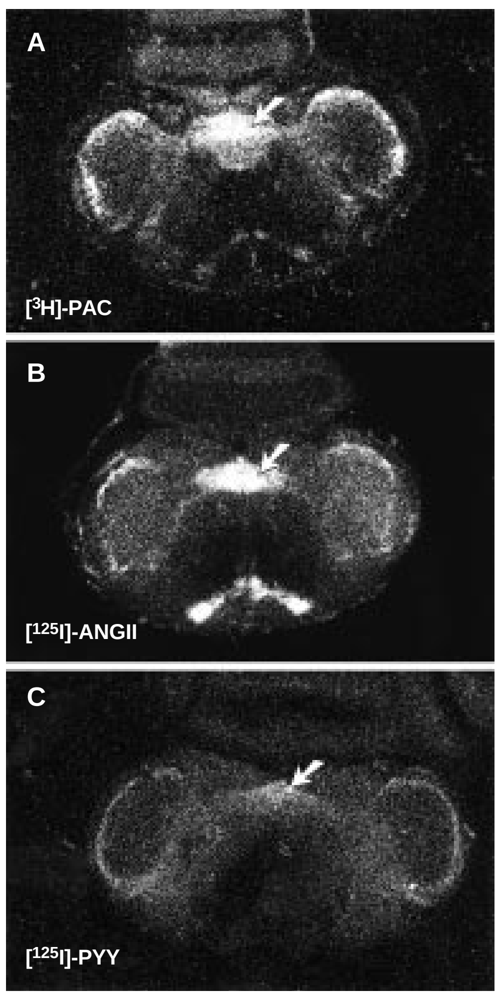

Figure 1 - Autoradiograms of coronal sections of the medulla oblongata of the rat showing the binding of different ligands in the nucleus tractus solitarii (arrow). *A*, Binding of [^{3}H]-p-aminoclonidine ([^{3}H]-PAC). *B*, Binding of [^{125}I]-angiotensin II ([^{125}I]-Ang II). *C*, Binding of [^{125}I]-neuropeptide Y ([^{125}I]-PYY).

Furthermore, interactions between the NPY receptor and the α_2-adrenoceptor have been described in other systems. Illes and Regenold (52) showed that NPY increased the hyperpolarizing effect of α_2-agonists such as noradrenaline and UK 14304 on locus ceruleus cells but had no effect on (Met$_5$)-enkephalin or (D-Ala$_2$, D-Leu$_5$)-enkephalin at opioid µ-receptors. These findings are in agreement with ours and suggest that NPY inhibits the action of catecholamines via a receptor-receptor interaction. Tsuda and collaborators (53) also suggested an interaction between NPY/α_2 adrenergic receptors since NPY inhibited the stimulation-evoked [^{3}H]-norepinephrine release in slices of the medulla oblongata.

These findings suggest the existence of receptor-receptor interactions involving the α_2-adrenoceptor and the NPY and Ang II receptors. These neurotransmission systems are involved in the mechanisms of blood pressure control and these interactions may have a role in the integration of extracellular signals in the central control of blood pressure. Actually, the receptor-receptor interactions among these neurotransmitter systems are altered in the NTS of spontaneously hypertensive rats (54,55), highlighting the possible participation of these interactions in the central mechanisms controlling blood pressure.

Although the presence of receptor-receptor interaction has been identified in several systems, the exact mechanism of this phenomenon has not yet been elucidated. A probable mechanism involves the participation of the G protein. The reduction of affinity of the α_2-adrenoceptors induced by NPY in membranes of the medulla oblongata was blocked by pretreatment with pertussis toxin (56). The treatment with pertussis toxin was also able to counteract the cardiovascular actions of both NPY and clonidine administered to the intraventricular system of the rat (57). The G protein seems to be also important in the modulation of α_2-adrenoceptors by bradykinin in the NTS (58).

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