DOI 10.1007/s00702-005-0279-3
J Neural Transm (2005) 112: 1309–1319

Journal of Neural Transmission
Printed in Austria

Formalin hindpaw injection induces changes in the [^{3}H]prazosin binding to α_1-adrenoceptors in specific regions of the mouse brain and spinal cord

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Received August 4, 2004; accepted January 8, 2005
Published online February 22, 2005;

Summary. Involvement of the α_1-adrenoceptor subtypes in early and late phases of formalin pain was investigated by quantitative *in vitro* autoradiography in the spinal cord and brain structures of CD-1 mice. Total α_1-adrenoceptors binding (including all α_1-adrenoceptor subtypes) was assessed with [^{3}H]prazosin; α_{1B}-adrenoceptor was assessed with [^{3}H]prazosin in the presence of 10 nM WB4101 to mask remaining α_1-adrenoceptor subtypes. Early after formalin injection the α_1-adrenoceptors (mainly α_{1B} receptor) binding was reduced in the contralateral hind limb area of the somatosensory cortex and in the secondary motor cortex. A reduction occurred also in the ipsilateral laminae I–III of the spinal cord (both α_{1B}- and non-α_{1B}-adrenoceptors). Lately an increase of α_1-adrenoceptors binding (mostly subtypes other than α_{1B}) appeared in discrete amygdaloid and thalamic nuclei. These results provide the first description of changes at the level of central α_1-adrenoceptors' binding during the formalin-induced pain in mice. Their distribution suggests that they may have a functional meaning.

Keywords: Alpha-1-adrenoceptor subtypes, [^{3}H]prazosin autoradiography, central nervous system, formalin pain.

Introduction

The descending noradrenergic and serotonergic pathways form a crucial link in the supraspinal modulation of nociceptive transmission (Fasmer et al., 1986; Dumka et al., 1996; Omote et al., 1998). The extensive studies on the involvement of noradrenergic system in pain and analgesia, carried out since the 70th (see Fürst, 1999), have demonstrated well its importance in pain modulation, as

exemplified by the observations that the disruption of the noradrenergic transmission (Kuraishi et al., 1983) and administration of noradrenergic antagonists (Fang and Proudfit, 1998) blocks or reduce the analgesic effects induced by administration of opiates.

Adrenoceptors that mediate the action of the endogenous noradrenaline are the members of G-protein-coupled receptor superfamily and belong to three distinct classes, named α_1-, α_2- and β-adrenoceptors, each one comprising several subtypes (see Nicholas et al., 1996). The α_1-adrenoceptors that are coupled with $G_{q/11}$ proteins and regulate calcium channels and phosphatydylinositol signaling cascade comprise three subtypes: α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors, which are encoded by separate genes, have distinct pharmacological profiles (see Michel et al., 1995; Zhong and Minneman, 1999), and are differently distributed (Price et al., 1994). The comparison of the distribution of the mRNAs coding for α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in the rat brain and spinal cord suggests unique functional roles for each of these receptors (Day et al., 1997). Although little is known about the α_1-adrenoceptor functions in the brain, their distribution pattern suggests that the α_{1A}-adrenoceptor is involved in thalamocortical sensory integration and participates in processes of learning and memory, while α_{1B}-adrenoceptor seems to participate in thalamocortical motor integration (McCune et al., 1993).

Most studies on noradrenaline-mediated effects in pain modulation have focused on the role of α_2-adrenoceptors since it has been observed that the activation of pre- and postsynaptic receptors of this type by noradrenaline released in the spinal cord from bulbospinal pathways is mainly responsible for inhibition of pain transmission (Li et al., 2000). Studies on the involvement of α_1-adrenoceptor in pain transmission were much less numerous. Almost all of them were basing on the results of administration of prazosin or other subtype-unspecific α_1-adrenoceptor antagonists, and conclusions were drawn basing on behavioral results (Kanui et al., 1993; Lee et al., 2000; Korzeniewska-Rybicka and Plaznik, 2001).

One of the widely used procedures to induce prolonged, tonic pain is the formalin test, in which mice receive intraplantar injection of formalin solution. The noxious response, regarded as the consequence of inflammatory processes, shows two distinct phases, early and late, differing in the mechanism of action and responsiveness to drugs (Porro and Cavazzuti, 1993).

The present study was undertaken to clarify the involvement of α_1-adrenoceptor, particularly its subtype α_{1B}, in the modulation of inflammatory pain. This was investigated by autoradiographic measurement of α_1-adrenoceptor distribution and densities in the regions of brain and spinal cord during the early and late phase of formalin pain.

Materials and methods

Animals

Male CD1 mice weighing 30–35 g (Charles River, Como, Italy) were used. They were housed in groups of four in standard breeding cages ($27\times24\times13$ cm), with free access to food and water, and on a standard 12/12 h light-dark cycle (07:00–19:00 h). All experiments were carried

out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

Formalin-induced pain

Formalin was injected in a standard manner used to produce inflammatory pain (Borghi et al., 2002). Briefly, the mice were injected subcutaneously, using a Hamilton syringe, into the dorsal surface of the right hind paw with 20 µl of 5% formalin solution, or saline. All mice injected with formalin immediately started to lick or bite the injected paw, indicating that the procedure was painful. The period of the first 15 min was considered the early phase of the formalin pain, while the late phase referred to time between 15 and 45 min after formalin injection (Borghi et al., 2002). At the end of the early and late pain phase (15 and 45 minutes after the injection of formalin or saline, corresponding to the end of the early and late phase, respectively) mice were decapitated and their brains and spinal cords were dissected and stored over dry ice.

From each brain isolated from three mice of each group (saline or formalin) and of each phase (early or late) two neighboring sections were taken for binding analysis (6 sections/group/phase). Four neighboring sections of each spinal cord obtained from four mice of each group (saline or formalin) and of each phase (early or late) were taken for binding analysis (16 sections/group/phase).

Autoradiography of α_1-adrenoceptors

Brain and spinal cord, dissected and frozen on dry ice, were used for the autoradiography of α_1-adrenoceptors. Frozen brain tissue was mounted on tissue holders and ten consecutive series of 12-µm thin coronal sections were taken across at 250-µm intervals on a Shandon cryostat (U.K.). Similar sections were also cut from the lumbar segment (L4 to L5) of the spinal cord. The sections were stored at $-70°C$. At the time of the assay, slide-mounted sections were thawed and preincubated for 1 h at room temperature in a Krebs modified buffer (KRBM) containing 10 mM Na_2HPO_4, pH 7.8; 119 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, and 1.3 mM $CaCl_2$. For the α_1-adrenoceptor assay, sections were incubated for 1 h with 0.9 nM [^{3}H]prazosin (Amersham; 25 mCi/mmol) either alone or with 10 nM WB4101. Adjacent sections were incubated with radioligand plus 10 µM WB4101 to determine nonspecific binding. According to Blendy et al. (1990) at concentration of 10 nM WB4101 occupies preferentially α_{1A}-adrenoceptors (86%), thus allowing assessment of α_{1B}-adrenoceptor subtype. The affinity of WB4101 to α_{1D}-adrenoceptor is similar to that of α_{1A}-adrenoceptor (Kenny et al., 1995), and therefore it may be assumed that at the used WB4101 concentration both α_{1A} and of α_{1D}-adrenoceptor be populations are masked and the binding parameters of α_{1B}-adrenoceptor may be specifically assessed.

Following incubation, sections were rinsed twice for 3 sec and 4 times for 10 min with ice-cold KRBM and then dipped briefly into ice-cold water. After washing, slides were rapidly dried with cool, dry air and left overnight. Slides were then apposed to 3H-Hyperfilm (Amersham) along with tritium standards and kept at room temperature for 2 months. Quantification of signals on the ^{3}H-Hyperfilm was performed using MCID (Microcomputer Imaging Device, Imaging Research, Brock University, Canada) software. Quantitative densitometry was carried out by calibration to a set of standards before reading the density values in the regions of interest. The ^{3}H isotope was used as reference. Optical density was converted to fmol/mg of tissue by comparison with a standard curve constructed from optical densities of tritium standards, using the specific activity of the radioligand.

Brain areas were identified by comparing autoradiographic images with appropriate plates from the mouse brain atlas of Franklin and Paxinos (1997). Several spinal, subcortical and cortical areas were considered, and are listed in caption to Fig. 1.

α_1-Adrenoceptor ligands

[^{3}H]prazosin was obtained from Radioactive Centre Amersham (25 mCi/mmol) and 2-([2,6-dimetoxyphenoxy-ethyl]aminomethyl)-1,4-benzodioxane (WB4101) from Sigma (St. Louis, MO, USA).

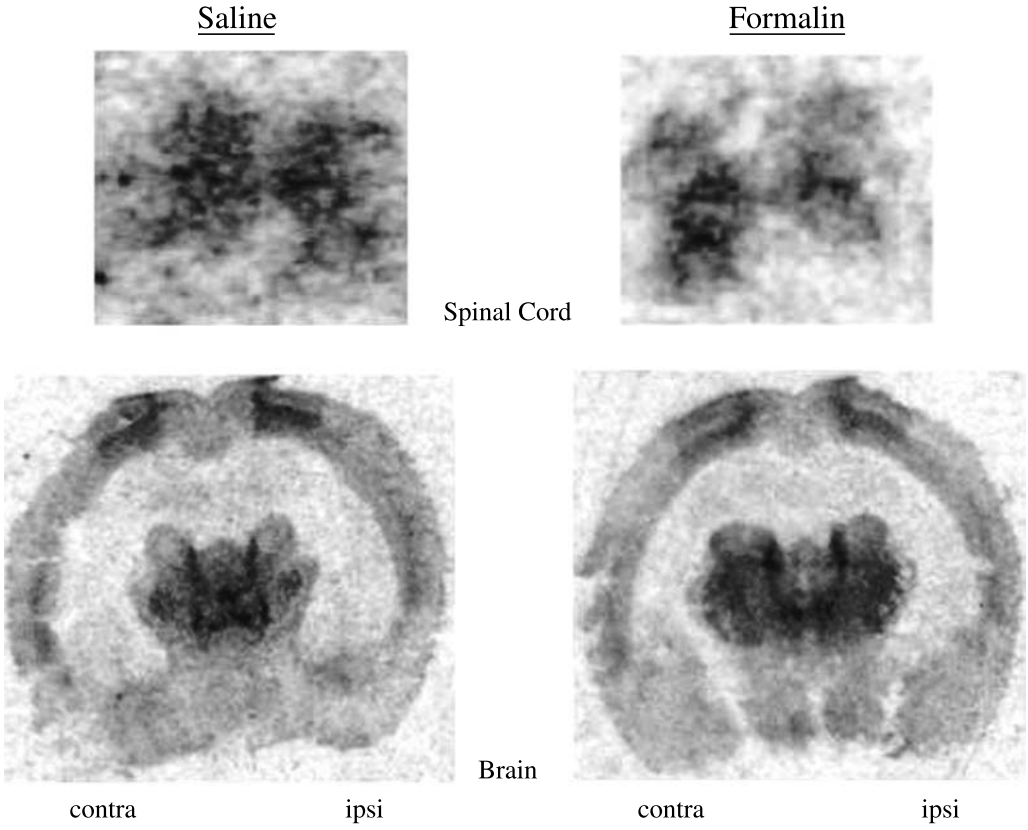

Fig. 1. Example of autoradiographic images of [^{3}H] prazosin binding 15 min (Spinal cord) and 45 min (Brain) after s.c. injection of saline or formalin into the right hind paw of the mouse. *Spinal cord* L4–L5 regions; *Brain* section equivalent to Franklin and Paxinos (1997) plate 45. *Contra–Ipsi* the sides contra- and ipsilateral to the injection site, respectively

Data analysis

The specific [^{3}H]prazosin binding was considered as indication of total α_1-adrenoceptor subtype binding, while the [^{3}H]prazosin binding in the presence of WB4101 (10 nM) – as the specific indication of α_{1B}-adrenoceptor subtype. The densities at the sites contralateral and ipsilateral to the injection site were compared, (save for the single central areas) as well as the difference between control (saline) and formalin injected subjects.

Autoradiographic data (expressed as fmol/mg of tissue) were analyzed (separately for each structure and receptor subtype) by three way analysis of variance, with treatment (formalin or saline), phase (early or late) and the injection side (contra- or ipsilateral) as factors or by two-way analysis of variance, with treatment (formalin or saline) and phase (early or late) in case of centrally located structures measured without division in ipsi and contraleteral side. The individual differences were subsequently tested for significance with the Fisher's LSD test if main effects or interactions were significant ($p < 0.05$).

Results

The formalin treatment produced discrete, often lateralized changes of α_1-adrenoceptor binding in several areas of the brain and spinal cord. The changes were different in the early phase, when there were predominantly decreases in the receptor binding, and in the late phase, when increases were predominantly

Table 1. Effect of formalin injection on binding of [^{3}H]prazosin (fmol/mg ± SEM) in the presence and absence of a 10 nM WB4101 measured in selected brain and spinal cord (L4–L5) regions

Structures	[^{3}H] Prazosin				[^{3}H] Prazosin + 10 nM WB4101			
	Ipsi		Contra		Ipsi		Contra	
	Control	Formalin	Control	Formalin	Control	Formalin	Control	Formalin
Early phase								
Spinal C (I–III)	33.4 ± 4.2	23.9 ± 1.4**	31.5 ± 3.6	26.2 ± 0.9	24.0 ± 1.2	19.7 ± 1.3*	21.0 ± 1.4	19.1 ± 1.6
Spinal C (X)#	35.7 ± 5.6	39.7 ± 3.1			22.7 ± 5.6	23.1 ± 1.5		
Thal CL	70.7 ± 2.2	70.7 ± 2.5	74.6 ± 3.2	72.9 ± 2.5	58.5 ± 1.7	60.6 ± 1.7	55.3 ± 4.8	61.5 ± 2.1
Thal PC	74.0 ± 3.7	66.6 ± 2.0	72.9 ± 5.5	67.3 ± 2.4	66.0 ± 2.9	55.9 ± 3.0	54.1 ± 1.0	57.6 ± 2.8
Thal CM#	78.0 ± 2.7	73.5 ± 3.6			66.6 ± 3.1	61.6 ± 5.0		
Amy CeC	28.0 ± 2.8	28.2 ± 1.5	23.7 ± 2.2	29.5 ± 1.4	19.2 ± 2.9	17.6 ± 1.4	16.4 ± 2.0	18.1 ± 1
Amy LaDL	23.7 ± 1.4	29.7 ± 0.8	26.3 ± 0.8	27.6 ± 3.4	15.9 ± 2.1	18.8 ± 1.2	17.3 ± 1.5	16.5 ± 2.7
Amy LaVL	26.3 ± 2.5	28.0 ± 1.2	24.9 ± 1.6	33.6 ± 2.3*	20.8 ± 2.2	18.8 ± 0.6	21.3 ± 3.3	20.1 ± 1.7
Cortex M1	35.6 ± 3.5	35.0 ± 1.9	44.6 ± 4.2	36.3 ± 2.9	27.4 ± 1.4	24.3 ± 2.2	32.7 ± 2.9	24.7 ± 2.7
Cortex M2	40.2 ± 2.5	33.3 ± 1.8	49.2 ± 2.9	34.4 ± 3.3*	31.2 ± 0.8	28.2 ± 2.6	38.2 ± 1.5	26.4 ± 2.9**
Cortex HL	47.3 ± 5.9	38.0 ± 2.0*	47.4 ± 3.7	37.7 ± 2.6*	26.7 ± 2.7	31.6 ± 1.8	32.9 ± 2.0	25.8 ± 3.1*
Late phase								
Spinal C (I–III)	33.9 ± 1.8	37.4 ± 2.1	29.1 ± 3.8	31.3 ± 2.4	20.7 ± 1.2	22.9 ± 2.1	18.1 ± 1.0	16.8 ± 1
Spinal C (X)#	36.9 ± 1.3	31.7 ± 1.5			28.9 ± 1.4	24.7 ± 1.4		
Thal CL	70.7 ± 2.5	88.1 ± 3.8**	82.2 ± 7.4	83.6 ± 4.4	64.9 ± 5.4	60.1 ± 6.7	58.9 ± 7.8	59.8 ± 6.6
Thal PC	76.7 ± 5.7	79.4 ± 3.7	79.4 ± 7.8	74.4 ± 5.0	64.6 ± 5.9	70.4 ± 5.8	52.3 ± 5.3	55.6 ± 9.2
Thal CM#	85.1 ± 6.6	95.8 ± 5.2			62.8 ± 9.3	81.8 ± 3.7*		
Amy CeC	33.8 ± 0.9	29.1 ± 2.2	23.9 ± 2.0	38.1 ± 2.7**	19.9 ± 1.7	20.4 ± 3.7	14.8 ± 3.0	22.3 ± 3.3*
Amy LaDL	26.0 ± 1.1	35.7 ± 2.6**	29.9 ± 1.6	42.0 ± 2.9**	18.9 ± 4.7	23.1 ± 4.6	18.9 ± 3.4	28.0 ± 6
Amy LaVL	34.8 ± 3.8	36.9 ± 3.7	32.7 ± 2.2	39.1 ± 3.2	21.6 ± 2.8	25.0 ± 4.1	21.1 ± 3.5	26.6 ± 5.5
Cortex M1	37.2 ± 3.1	33.7 ± 4.2	42.0 ± 3.3	41.5 ± 7.8	24.3 ± 3.4	26.6 ± 3.0	26.4 ± 5.0	27.0 ± 1.6
Cortex M2	34.3 ± 4.3	41.4 ± 7.7	45.1 ± 4.5	42.1 ± 5.5	33.3 ± 5.3	26.1 ± 2.3	33.2 ± 3.6	29.6 ± 3.4
Cortex HL	45.8 ± 2.4	26.7 ± 1.1**	44.5 ± 1	28.6 ± 1.1**	16.6 ± 1.3	23.5 ± 3.0	23.3 ± 1.7	22.5 ± 2.4

Binding was done with [^{3}H]prazosin alone (to label all subtypes of α_1-adrenergic receptors) or in the presence of 10 nM WB4101 mask (to label α_{1B} only). Data are means ± SEM (n = 6) in the early and late phases of the formalin test. * $p < 0.05$ and ** $p < 0.01$ versus corresponding saline-injected control groups. # centrally located structures measured without division in ipsi and contralateral side. Abbreviations: *Spinal C* Spinal cord: Laminae I–III and Lamina X. *Thal* thalamic nuclei: *CL* central lateral; *PC* paracentral; *CM* central medial. *Amy* amygdaloid nuclei: *CeC* central nucleus analyzed together with the anterior part of the basolateral nucleus (BLA); *LaDL* lateral nucleus, dorsolateral part; *LaVL* lateral nucleus, ventrolateral part. *M1* primary motor cortex. *M2* secondary motor cortex. *HL* somatosensory cortex, hindlimb area

Table 2. Summary of main significant changes in [^{3}H]prazosin binding induced in central nervous system by formalin pain (early and late phases)

Subtypes of α_1-adrenoceptor	non-α_{1B}		α_{1B}	
Brain area/Side	Contra	Ipsi	Contra	Ipsi
Early phase				
Spinal cord (I–III)		↓↓		↓
Amy LaVL	↑			
Cortex M2	↓		↓↓	
Cortex HL	↓	↓	↓	
Late phase				
Spinal cord (I–III)				
Thal CL		↑↑		
Thal CM[#]				↑
Amy CeC	↑↑		↑	
Amy LaDL	↑↑	↑↑		
Cortex M2				
Cortex HL	↓	↓		

Up and down arrows indicate respectively, the increase and decrease of the binding observed in the spinal cord and brain areas. [#] Centrally located structure. *Contra–Ipsi* the sides contra- and ipsilateral to the site of formalin injection, respectively. Abbreviations, see Table 1

observed (Tables 1 and 2). Example of autoradiographic image showing the distribution of α_1-adrenoceptors in the brain and lumbar spinal cord during the two phases induced by formalin pain is presented in Fig. 1.

Changes in the binding of α_{1B}-adrenoceptor induced by formalin injections

Early phase

Analysis of individual differences revealed that at the early phase of the formalin action α_{1B}-adrenoceptor binding (Table 1, right side) was depressed (by 18%, $p < 0.05$) in the laminae I, II, III of the spinal cord (laminae) ipsilateral to the injection site. More pronounced were the decreases in the contralateral motor cortex (by 30%, $p < 0.01$) and in the part of somatosensory cortex related to the hind limb area (by 22%, $p < 0.01$).

Late phase

The effects observed in the early phase disappeared in the late phase, but changes in the opposite direction – elevation of the α_{1B}-adrenoceptor – appeared in subcortical structures. Those structures were the contralateral central amygdaloid nucleus (increase by 50%) and the centromedial thalamic nucleus (by 30%).

The three-way analysis of variance showed: a significant effect of side [$F_{(1/120)} = 9.02$, $p < 0.01$] with no significant effect of formalin and phase in the spinal cord (laminae I–III), a significant effect of formalin [$F_{(1/40)} = 8.48$,

$p<0.01$] without a significant effect of phase and side in the motor cortex (M2), and significant effects of formalin [$F_{(1/40)}=41.82$, $p<0.01$] and phase [$F_{(1/40)}=8.79$, $p<0.01$] in the somatosensory cortex (HL).

Changes in the total binding of α_1-adrenoceptor induced by formalin injections

As it was impossible to directly label non-α_{1B}-adrenoceptors, an indirect approach was undertaken: measuring of the total α_1-adrenoceptors binding, and drawing conclusion from the differences between the results of total and specific α_{1B}-adrenoceptor binding.

Early phase

The changes of the total binding to α_1-adrenoceptor subtypes (Table 1, left side) in the early phase followed the effects found for the α_{1B} subtype, but while they were similar in size in the cortical areas, they were much more expressed in the external laminae of the spinal cord (a decrease by 29%, $p<0.05$). This suggests that the non-α_{1B} α_1-adrenoceptors are specifically affected by formalin injection. The three-way analysis of variance of the results from the spinal cord revealed that formalin acted here differently in the early and late phases of formalin pain (the significant effect of phase [$F_{(1/120)}=4.42$, $p<0.05$] with significant interaction formalin $\times$ phase [$F_{(1/120)}=6.7$, $p<0.01$]).

In the cortical areas a specific depression of non-α_{1B}-adrenoceptors in the ipsilateral hind limb related somatosensory cortex was suggested, as the total binding to α_1-adrenoceptors was decreased there by 20% ($p<0.05$), while no changes in the α_{1B}-adrenoceptor binding were noted (the three-way analysis of variance showed the significant effect of formalin [$F_{(1/40)}=41.82$, $p<0.01$] and phase [$F_{(1/40)}=8.79$, $p<0.01$]). In a pain-related subcortical structure, the ventrolateral amygdaloid nucleus, in contrast to the cerebral cortex, an increase rather than decrease in non-α_{1B}-adrenoceptors (by 35%, $p<0.05$) was observed. (The three-way analysis of variance showed the significant effect of formalin [$F_{(1/40)}=6.49$, $p<0.05$] and phase [$F_{(1/40)}=16.92$, $p<0.01$]).

Late phase

In the late phase similarly as in the early phase, the non-α_{1B}-adrenoceptors were decreased in the hind limb related somatosensory cortex (approx. by 40% on both sides, $p<0.01$). The other changes observed in the early phase disappeared, but instead increases in non-α_{1B}-adrenoceptors appeared in the specific nuclei of the thalamus and amygdala. Thus the elevations of non-α_{1B}-adrenoceptors were observed in the thalamic ipsilateral central lateral nucleus, (by 25%, $p<0.01$; significant effect of phase, $F_{(1/40)}=10.12$, $p<0.01$), the contralateral central amygdaloid nucleus (by 59%, $p<0.01$; significant effect of formalin [$F_{(1/40)}=6.82$, $p<0.05$] and phase [$F_{(1/40)}=6.83$, $p<0.05$], with interaction formalin $\times$ side [$F_{(1/40)}=16.64$, $p<0.01$] and formalin $\times$ phase $\times$ side [$F_{(1/40)}=5.05$, $p<0.05$]), and the dorsolateral amygdaloid nucleus (approx. by 40% on both sides, $p<0.01$; significant effect of formalin [$F_{(1/40)}=23.94$,

$p < 0.01$]; significant effect of phase [$F_{(1/40)} = 19.37$, $p < 0.01$] and interaction formalin × phase [$F_{(1/40)} = 5.88$, $p < 0.05$]).

Discussion

α-Adrenoceptors, which are widely dispersed in the brain, were proven to be involved in pain control (see Fürst, 1999). While α_2-adrenoceptors mediate antinociception, the activation of supraspinal α_1-adrenoceptors is pronociceptive (Kingery et al., 2002). The pronociceptive role of α_1-adrenoceptors may be attributed to their propensity to induce an increase in the intraneuronal calcium, whose pronociceptive role is well documented (see Miller, 1987).

Two distinct subtypes of α_1-adrenoceptors, α_{1A} and α_{1B} exist in the central nervous system, and the pattern of the distribution of mRNA coding for them (Day et al., 1997) is roughly correlated with their regional expression, as shown in this paper (Fig. 1, Table 1).

Both α_{1A}- and α_{1B}-adrenoceptor subtypes serve to initiate the calcium signal in the cell, though the mechanisms of their action may differ (see Zhong and Minneman, 1999, for review). It is of interest whether the both subtypes affect the nociception by a similar or different mechanisms. However, using subtype-unspecific α_1-adrenoceptor antagonists, such as prazosin, it is impossible to answer that question. We tried, therefore, to approach this problem by investigation, by means of autoradiography, whether pain induces changes in the density of various receptor subtypes. Our strategy consisted of masking of α_{1A}- and α_{1D}-adrenoceptors with low concentration of WB4101 to allow investigating the changes in α_{1B}-adrenoceptor subtype, as described in the Methods. This approach was presently used in the model of tonic inflammatory pain induced by formalin. Formalin pain is induced and maintained in specific projection areas in the somatosensory cortex. The areas have well-defined transmission pathways and are lateralized.

The formalin pain is a complicated, biphasic phenomenon (Porro and Cavazzuti, 1993). Its early phase is related to the activation of nociceptive terminals close to the site of injection of the noxious agent, while the late phase is caused by more complex inflammatory reactions. The pain mechanisms involved in those two phases are thus different, and this is reflected by the fact that the effectiveness of various agents in modulation formalin pain in various phases is different. Thus, e.g., systemically injected prazosin antagonizes only the early phase of formalin pain (Tasker et al., 1992; our unpublished results). This difference in action of prazosin is related to its central, and not local peripheral effects, as shown by the fact that when given into the formalin-injected paw, it is equally effective in both early and late phase of formalin pain (Hong and Abbott, 1996). Our present results show that in some specific areas of the central nervous system formalin pain induces changes of α_1-adrenoceptor binding. Those changes in many cases are lateralized, are different in the early and late phase of pain, and concern specific subtypes of α_1-adrenoceptor.

In the acute phase formalin induced a reduction of both α_{1B}- and remaining α_1-adrenoceptors in the ipsilateral superficial laminae of the lumbar spinal cord, suggesting their involvement in the pain transmission via the anterolateral

system. As the effect was much more pronounced when all α_1-adrenoceptor subtypes were visualized, it indicates relatively minor role for α_{1B}-adrenoceptors in this case. In contrast, the potent decreases in the α_{1B}-adrenoceptor observed in the somatosensory cortex containing projections from the hind limb and in the secondary motor cortex, equal to the decreases observed in the samples labeled for all α_1-adrenoceptor subtypes, suggest that the involvement of α_{1B}-adrenoceptors in these areas is of considerable importance. Although the meaning of those changes is presently not clear, it is worth noticing that they occurred specifically in areas involved in pain signaling or processing. The changes in the secondary motor cortex are probably related to the change in locomotor activity in adaptation to pain in the hind limb (intensive paw shaking, licking, and biting). Interestingly, the binding to α_1-adrenoceptors other than α_{1B} is reduced also in the contralateral sensorimotor cortex, suggesting that, in contrast to α_{1B}-adrenoceptors, they are not directly limited to the receptor field.

The changes in the late phase of formalin pain are much more difficult to interpret. As generally they do not affect the areas involved in the early phase indicates that the pain mechanisms in both phases are different. The fact that the density of α_{1B}-adrenoceptors increased in the nuclei of amygdaloid complex may suggest that they are related rather to emotional aspects of pain or to pain-induced aggressiveness, in which amygdala is involved (Gregg and Siegel, 2001). The α_1-adrenoceptors other than α_{1B} are affected in specific subcortical regions, when the increases in the binding are invariably noted, while the changes in the sensorimotor cortex are similar to those observed in the early phase of pain. Interestingly, these changes are predominantly bilateral, suggesting that in subcortical structures they may represent generalized, indirect responses with a marked emotional component.

The mechanism by which the changes in α_1-adrenoceptor density are effected is unclear. The differences in labeling may be due to changes in affinity of those receptors or the result of changing the proportion of internalized and non-internalized receptors. Regardless of the mechanism of changes one may assume simplistically that a decrease in labeling represents the reduction of function (down-regulation) and vice versa. Using such an assumption we suggest that noradrenergic receptors in sensory cortex are suppressed in both phases of formalin pain. This may be regarded as a rapid adaptation aimed at dampening of nociceptive mechanisms, and therefore to reduce the excessive central perception of pain. It is plausible that the down-regulation is related to an increase in noradrenaline level, as the pain brought about by an intraplantar formalin injection induces a several fold increase in noradrenaline level in blood plasma (Culman et al., 1997), in the hypothalamic paraventricular nucleus (Palkovits et al., 1999) and in the spinal cord (Omote et al., 1998).

Formalin injections affected also the α_1-adrenoceptors binding in amygdalar nuclei, involved in emotional response to pain and possibly to pain-induced aggressiveness. The up-regulation of α_1-adrenoceptors (other than α_{1B} subtype) in these structures may correlate with enhanced emotional responses induced by chronic pain. The emotional response is not immediate, and accordingly the changes are observed predominantly in the late phase. In addition, our data suggest that different subtypes of α_1-adrenoceptors are involved in the early

and the late phase of pain. The increase of the binding of α_1-adrenoceptors in the late phase were attributed mostly to the other than α_{1B} subtype, and this corresponds well with behavioral data showing that the α_{1A} subtype blockade attenuates the second phase of pain (Hong and Abbott, 1996).

A question may arise whether the observed changes induced by formalin injection are related to the central mechanisms of formalin-induced pain? Without insisting on the idea that the changes observed in [^{3}H]prazosin binding in brain areas are related to pain perception we demonstrated that the changes appeared in the structures known to be involved in pain perception (see Rainville, 2002). Moreover, their lateralization in some of the areas and the lack of lateralization in the others are in agreement with our knowledge about the course of pain signaling pathways. Therefore the data strongly support the view that the binding changes are related to nociceptive effects of formalin.

Summing up, our results demonstrate that α_1-adrenoceptors are involved in the formalin nociception, and suggest that they are down-regulated to reduce both the processing of pain signaling in the spinal cord (in the early phase) and perception in the sensorimotor cortex (in both early and late phase). Moreover, their up-regulation in the late phase of formalin pain in the nuclei of amygdaloid complex may be related to behavioral consequences of chronic pain. We also have shown that the α_{1B}-adrenoceptors in certain brain areas and phases of the formalin pain respond differently than other subtypes of α_1-adrenoceptor, being particularly involved in the early responses in cortical structures.

Acknowledgements

This paper originated in the frame of cooperation between the Italian National Research Council and the Polish Academy of Sciences. The work was partly supported by the statutory funds of the Institute of Pharmacology, Polish Academy of Sciences.

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