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Physiological and pathological role of the constitutively active α_{1D} adrenoceptors

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Abstract

In previous studies we have shown that in vessels where α_{1D} adrenoceptors have a functional role, this subtype exhibits constitutive activity, revealed by an increase in contractile tone, cytosolic calcium and inositol phosphate accumulation, in the absence of an agonist. The contraction and the two internal signals were inhibited by prazosin and BMY 7378, a selective α_{1D} adrenoceptor ligand. This constitutively active population of α_{1D} adrenoceptors plays a modulatory role in conductance vessels, preventing abrupt changes in vessel caliber, and consequently, in blood flow, when the stimulus disappears. The lack of this subtype in distributing arteries warrants a quick and fine adjustment of blood flow to adrenergic stimulus. An increased functionality of α_{1D} adrenoceptors in aorta, mesenteric and small mesenteric arteries of spontaneously hypertensive adult rats (SHR) has been observed. Consequently, the contractile tone of these vessels remained increased when the adrenergic stimulus disappears as compared with normotensive animals. This change is evidenced only when the hypertensive state appears as it is not observed in prehypertensive (young rats), or captopril-treated adult animals, therefore, it could be directly related to hypertension.

Keywords: α_{1D} Adrenoceptors; Constitutive activity; Hypertension

1. Introduction

Molecular cloning techniques and pharmacological studies have identified the existence of three subtypes of α_1 adrenoceptors, α_{1A}, α_{1B} and α_{1D}, all of which are expressed in vascular smooth muscle and play a role in the regulation of blood pressure. However, the

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functionality of each subtype has not been well defined and the reason for the coexistence of different subtypes in the same tissue or for the different distribution of the subtypes between vessels remains unclear. We have shown in previous studies [1–7] that in vessels where α_{1D} adrenoceptors have a functional role, this subtype exhibits constitutive activity, revealed by an increase in the contractile tone. The assumption that this increase is due to the constitutive activity of α_{1D} adrenoceptors is based on the following evidences: (i) it is induced in the absence of the agonist, (ii) it is inhibited by the α_1 adrenoceptor ligand prazosin and the selective α_{1D} adrenoceptor ligand BMY 7378 and (iii) the irreversible α_1 adrenoceptor antagonist chloroethylclonidine, which inhibits noradrenaline-induced contractions in this tissue, does not affect the increase in the contractile tone but prevents the inhibitory effect of BMY 7378. In addition, two different groups have found constitutively active cloned α_{1D} adrenoceptors in stably transfected Rat-1 fibroblasts [8,9]. In the present work, we discuss the physiological and the pathological role of this population of constitutively active α_{1D} adrenoceptors in arterial vessels, on the basis of recent evidences about cellular localization of α_{1D} adrenoceptors, its coupling to internal signals (calcium and inositol phosphates) and its increased role during hypertension.

2. Discussion

2.1. Physiological role of the constitutively active α_{1D} adrenoceptors

Previous results obtained using a simple experimental procedure in contractility studies on isolated organ bath permit us to evidence the existence of a constitutively active population of α_{1D} adrenoceptors in rat aorta, iliac and proximal mesenteric arteries [2,5,6]. The procedure, which permits us to easily quantify the constitutive activity of α_{1D} adrenoceptors, is depicted in Fig. 1. Noradrenaline, by activating α_1 adrenoceptors, elicits a contractile response in Ca^{2+}-free medium (NA1 in Fig. 1), due to Ca^{2+} release from internal stores. A small or no contraction was evoked upon a second application of the agonist (NA2 in Fig. 1), which indicates depletion of internal Ca^{2+} stores sensitive to NA. The tissues were then incubated in Ca^{2+}-containing solution in absence of the agonist and an increase in the resting tone (IRT in Fig. 1) was observed in aorta, iliac and proximal mesenteric artery [2,5,6]. As has been analyzed and discussed in previous papers [1–5], endogenous or exogenous agonists were not present. Therefore, the fact that the IRT was inhibited by prazosin and BMY 7378, the selective antagonist of the α_{1D} subtype, indicates the existence of a population of α_{1D} adrenoceptors in a constitutively active state [2,5–7].

The fact that this IRT is closely related to α_1 adrenoceptors and not to internal calcium mobilization and/or to the emptying of intracellular Ca^{2+} pools is demonstrated by the depletion of internal Ca^{2+} stores by other α_1 adrenoceptor agonists such as methoxamine and phenylephrine which also elicits IRT, whereas clonidine, serotonin, caffeine, ryanodine, thapsigargin and cyclopiazonic acid, which deplete Ca^{2+} stores but not through activation of α_1 adrenoceptors, did not elicit any IRT [1,3,4]. Moreover, in aorta, iliac and proximal mesenteric artery, a functional population of α_{1D} adrenoceptors has been previously shown but in vessels such as tail or small mesenteric branches a population

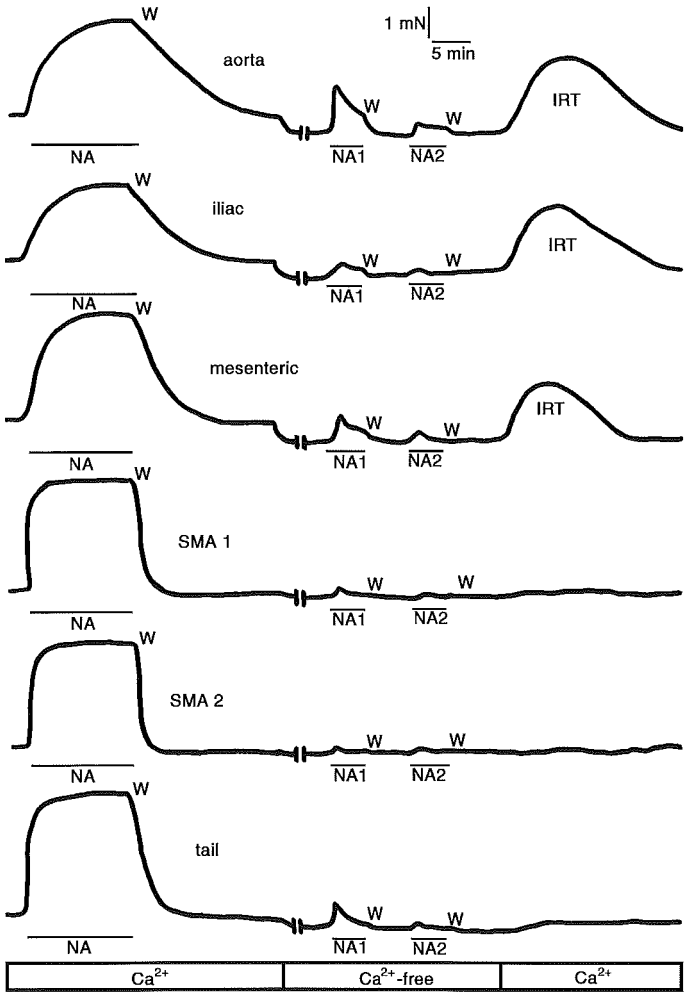

Fig. 1. Experimental procedure designed to evidence the constitutively active population of α_{1D} adrenoceptors in different vessels. Noradrenaline (NA) was added in Ca^{2+}-containing solution (Ca), and after washing (W) and recovery of the basal tone, the tissue was incubated for 20 min in Ca^{2+}-free, EDTA-containing solution (Ca^{2+}-free). After this time the agonist was applied (NA1, NA2) and washed. The tissue was then incubated for 20 min in Krebs and a spontaneous increase in the resting tone (IRT) of aorta, iliac and proximal mesenteric artery (not tail artery or first (SMA-1) or second (SMA-2) small mesenteric arteries) was observed.

of α_{1A} (α_{1L}?)—and/or α_{1B} but not α_{1D} adrenoceptors has been described [2,5,6,10–15]. The fact that IRT was not observed in tail and small mesenteric branches (Fig. 1) where α_{1D} adrenoceptors do not exhibit a functional role suggests that this constitutive activity is only shown by the α_{1D} adrenoceptor subtype. Interestingly, the same observation about the constitutive activity of α_{1D} adrenoceptors was reported by two different groups of researchers working with cloned α_{1D} adrenoceptors expressed in Rat-1 fibroblasts [8,9].

It is well known that α_1 adrenoceptors utilize a variety of second messenger pathways to modulate cellular function [16–18], including mobilization of intracellular Ca^{2+} as a consequence of the IP accumulation and activation of Ca^{2+} influx via voltage-dependent and -independent Ca^{2+} channels. Therefore, we analyzed the changes in cytosolic calcium content and inositol phosphate accumulation related to the constitutively active population of α_{1D} adrenoceptors.

In rat aorta, by activating α_1 adrenoceptors, noradrenaline elicits a sustained contraction that slowly decreases after washing the agonist, accompanied by an increase in the calcium content (Fig. 2) and an accumulation of inositol phosphates (Fig. 3). In Ca^{2+}-free medium, noradrenaline also induces a contractile response, IP accumulation and increase in the cytosolic calcium due to its release from internal stores (NA 1, NA 2 in Figs. 2 and 3); during posterior incubation in Ca^{2+}-containing solution in absence of the agonist, a spontaneous increase in the contractile tone was observed (Fig. 2) accompanied by a biphasic (phasic followed by tonic) increase in the internal calcium content (Fig. 2) and an increase in the inositol phosphate accumulation (Fig. 3). In tail artery, the same experimental procedure gives different results: no increase in contractile tone nor in inositol phosphate accumulation as observed during loading in calcium-containing medium and only a tonic but not a phasic increase in internal calcium content was observed (Figs. 2 and 3).

The three signals observed in aorta and in absence of noradrenaline, increase in tone, in Ca^{2+} content (the phasic component, not the tonic one) and in inositol phosphate accumulation, were inhibited by prazosin and BMY 7378 [7], relating them to α_{1D} adrenoceptor constitutive activity. Prazosin and BMY 7378 did not inhibit the tonic increase in $[Ca^{2+}]_i$ suggesting therefore that both components of calcium entry are pharmacologically distinguished. The lack of functional α_{1D} adrenoceptors in tail artery [5,13] is likely to be responsible for the absence of the phasic $[Ca^{2+}]_i$ increase, the inositol phosphate accumulation and the contractile response in this tissue [7].

It is interesting to note that IP accumulation derived from the constitutively active α_{1D} adrenoceptors only appeared when extracellular Ca^{2+} was restored [7]. However, noradrenaline-induced IP accumulation was not dependent on $[Ca^{2+}]_i$ because noradrenaline induced similar increases in IP accumulation in Ca^{2+}-containing (NA) and Ca^{2+}-free medium (NA1 and NA2) despite $[Ca^{2+}]_i$ levels being much lower in the latter conditions (Figs. 2 and 3). Therefore, and this is a crucial point, these results suggest that, in the absence of an agonist, the coupling of α_{1D} adrenoceptors with G proteins requires Ca^{2+}. In fact, Ni^{2+}, which almost suppressed the increase in $[Ca^{2+}]_i$, also abolished the IP accumulation upon restoration of Ca^{2+} [7].

An important question which these results raises is whether in native tissues the α_{1D} adrenoceptors are "*truly*" constitutively active if they need calcium to manifest their activity. As has been cited above, two different groups have found constitutively active α_{1D} adrenoceptors in stably transfected Rat-1 fibroblasts but their Ca^{2+} dependence has not been analyzed [8,9]. Moreover, according to previous papers [9,16,19], in native vascular smooth muscle, where two (or three) α_1 adrenoceptor subtypes coexist, the α_{1D} subtype is localized mainly intracellularly in a perinuclear orientation, whereas the other two are expressed in the cell membrane. It is well known that an agonist binds to α_{1A} or α_{1B} receptors in the membrane and these receptors couple to G-proteins inducing a

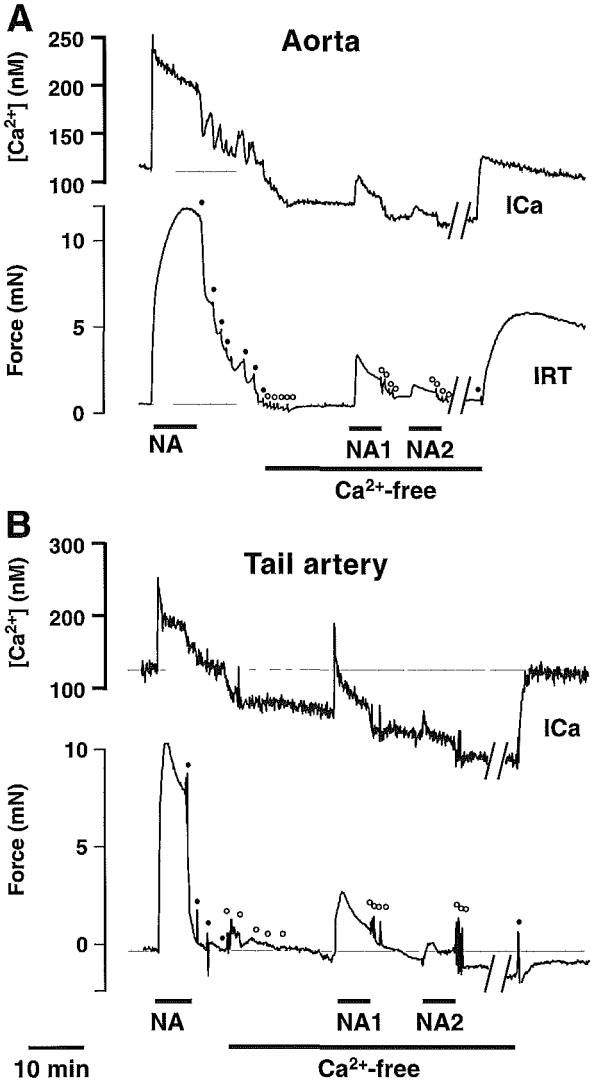

Fig. 2. Representative tracings of simultaneous recordings of $[Ca^{2+}]_i$ and contractile force during the experimental procedure used to study the constitutively active population of α_{1D} adrenoceptors in fura-2 preloaded aorta and tail artery. Noradrenaline (1 μM in aorta and 10 μM in the tail artery) (NA) was added and the sustained responses were taken as a control. After washing and recovery of the basal tone, tissues were incubated for 20 min in Ca^{2+}-free solution and then noradrenaline was added (NA1). Afterwards, the tissues were washed in Ca^{2+}-free solution and exposed to noradrenaline (NA2). Arteries were then incubated for 20 min in Ca^{2+}-free solution and then extracellular $CaCl_2$ was restored which raised $[Ca^{2+}]_i$ and induced a contractile response only in the aorta (denoted by IRT). The dotted line represents the baseline and the small solid and open circles indicate washouts in Ca^{2+}-containing and Ca^{2+}-free solution, respectively. (Data from Ref. [7].)

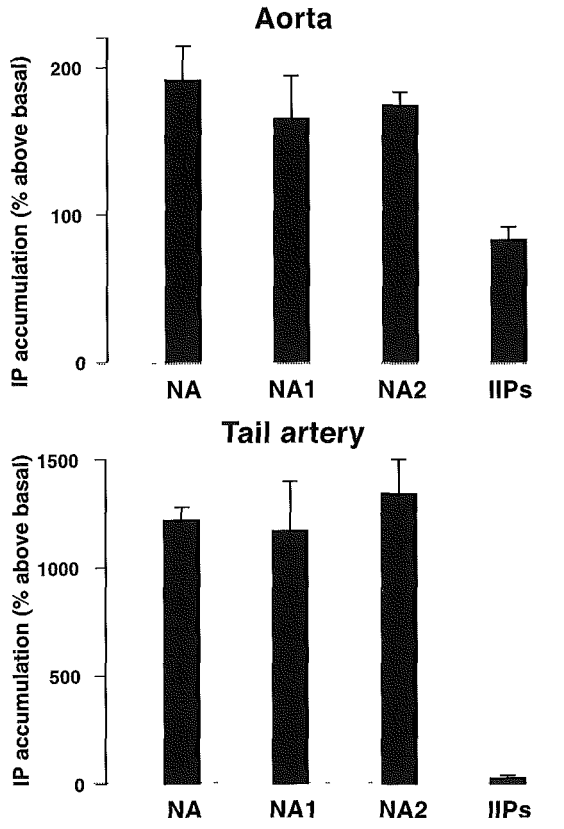

Fig. 3. Changes in IP accumulation in rat aorta and tail artery following the protocols shown in Fig. 1. NA indicates the effects of noradrenaline in Ca^{2+}-containing solution, NA1 and NA2 the effects of successive applications of noradrenaline in Ca^{2+}-free solution to deplete intracellular Ca^{2+}-stores and IIPs the effects of restoring extracellular Ca^{2+}. (Data from Refs. [5,7].)

response but they are then phosphorylated and internalized [16,19]. We propose that this internalization may be the signal for the migration of the constitutively active α_{1D} adrenoceptors to the membrane surface where they couple to G-proteins and maintain the response even when the agonist is removed; finally, they are also phosphorylated and internalized. The function of Ca^{2+} is to permit migration to the external membrane of α_{1D} adrenoceptors. This hypothesis explains some experimental data which are not completely understood, such as the existence of several adrenoceptor subtypes differently localized in the same cell and mediating the same response, but the more convincing argument in favour of our proposal is the fact that a native receptor constitutively active in a cell looses its functionality as a "receptor" of external signals. Therefore, its function must be regulated by these external signals in some way.

According to this hypothesis, the existence of a constitutively active population of α_{1D} adrenoceptors in large conducting vessels such as aorta, plays a modulatory role in their tone because the contractile response to an adrenergic stimulus can be sustained even

when the agonist is removed, thus, preventing abrupt changes in the vessel caliber, and consequently, sudden changes in blood flow. In contrast, the lack of a functional population of α_{1D} adrenoceptors in distributing vessels such as tail or resistance vessels such as small mesenteric branches warrants a quick, fine adjustment of contractile tone and blood flow according to the adrenergic stimulus. Confirming this proposal, we have shown that in vessels such as aorta, iliac and proximal mesenteric arteries where a functional population of α_{1D} adrenoceptors exist, the contractile response to NA slowly appeared (Figs. 1 and 2) and slowly disappeared when the agonist was removed (Figs. 1, 2 and 4). On the contrary and according to our proposal, in vessels such as tail, distal mesenteric and small mesenteric arteries where a functional population of α_{1D} adrenoceptors must be excluded, a faster contractile response to noradrenaline (Figs. 1 and 2) followed by a faster decrease in the tone after removal of the agonist (Figs. 1, 2 and 4) was observed.

2.2. Pathological role of the constitutively active α_{1D} adrenoceptors

According to the above, an imbalance in the modulating mechanism played by the constitutively active α_{1D} adrenoceptors could be involved in pathologies such as hypertension, in the pathogenesis and/or maintenance of which α_{1D} adrenoceptors play a role, as has been postulated by different authors [20,21].

In order to clarify this point, we analyzed the participation of the constitutively active α_{1D} adrenoceptors in arteries obtained from spontaneously hypertensive rats (SHR) and controls (WKY) divided into three groups: young prehypertensive (6 weeks old), adult hypertensive (16 weeks old) and adult animals chronically treated with captopril (50 mg kg^{-1} per day orally from 6 to 16 weeks) in order to prevent the hypertensive state [22].

In adult SHR animals, a significant increase in BMY 7378 potency (not in prazosin potency) was observed in some vessels such as aorta, mesenteric and small mesenteric arteries [22], suggesting an increased functional role of α_{1D} adrenoceptors in these vessels.

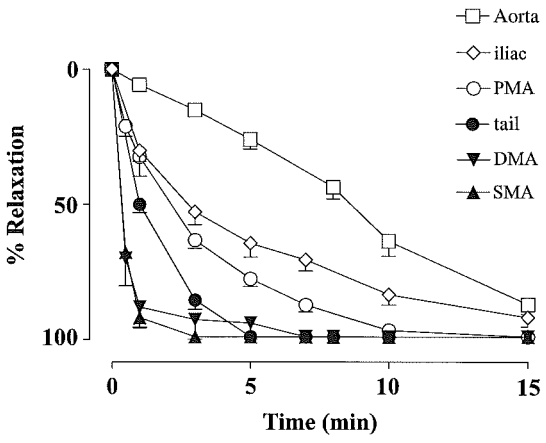

Fig. 4. Time course of the decay in the maximal contractile response to noradrenaline after removal of the agonist in aorta, iliac, proximal mesenteric artery (PMA), distal mesenteric artery (DMA), first branch of small mesenteric artery (SMA) and tail artery. (Data from Refs. [5,6].)

This difference was not observed in iliac or tail arteries. The increase in resting tone (IRT) observed in the absence of agonist and inhibited by BMY 7378, which represents the constitutively active α_{1D} adrenoceptors, was also significantly greater in aorta and mesenteric artery from adult SHR [22]. In young and captopril-treated adult animals, no differences between strains with respect to BMY 7378 potency, or IRT were observed [22]. The pathological consequence of this change in aorta, mesenteric artery and small mesenteric branches of hypertensive animals is the slower rate of recovery of the basal tone after removal of an adrenergic stimulus, and as a result, the contractile tone of these vessels remained increased when compared with normotensive animals when the agonist was removed (Fig. 5). This change is evidenced only when the hypertensive state appears since it is not observed in prehypertensive animals or captopril-treated animals, therefore, it could be directly related to hypertension [22]. We suggest that it could be involved in the genesis and/or maintenance of this pathology. Future analysis of the mechanisms controlling the functional expression of each subtype of α_1 adrenoceptors in a given vessel could provide the clue to an understanding of the mechanism that triggers the hypertensive state.

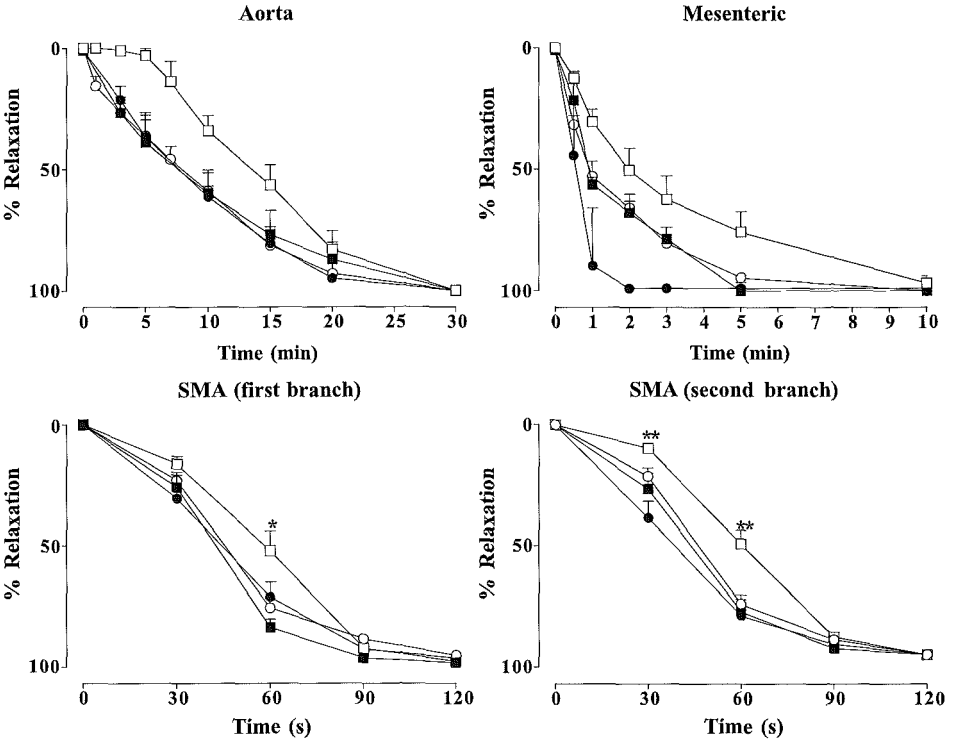

Fig. 5. Time course of the decay in the maximal contractile response to noradrenaline after removal of the agonist in aorta, proximal mesenteric artery, and first or second branch of the small mesenteric arteries (SMA) obtained from adult WKY (circles) or SHR (squares) animals pretreated (black symbols) or not (white symbols) with captopril (50 mg kg-1 per day orally) from age 6 to 16 weeks. (Data from Ref. [22].)

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Discussion 6

G. Milligan

One of the big problems with working with α_1 adrenoceptors is obviously the ligands for the pharmacology are not enormously selective. Have you tried to follow through some of this work by moving into mice with knockouts of the various subtypes?

P. D'Ocon

In the next months, we will try to confirm this hypothesis using α_{1D} adrenoceptor knockout mice.

M. Lohse

Is the affinity of noradrenaline the same for the two subtypes α_{1A} and α_{1D} adrenoceptors?

P. D'Ocon

No, it is not the same. The affinity of noradrenaline is higher for α_{1D} adrenoceptors.

M. Lohse

Could it be that the slower kinetics of the switch of reaction is related to this? You have a slower disassociation of noradrenaline from the receptor, so it appears constitutively active, but really you also have a higher proportion of agonist-occupied receptors compared to the α_{1A} adrenoceptor.

P. D'Ocon

I think it is not a problem of the noradrenaline affinity. We have an experiment in which we pre-incubate with a low concentration of the selective α_{1D} adrenoceptor ligand BMY 7378. In these conditions, we obtained a maximal response to noradrenaline but, after washing, the contraction immediately decayed. In addition, in the experiments with hypertensive animals, we observed a higher or lower rate of decay depending on the functionality of α_{1D} adrenoceptors.

M. Lohse

However, in your hypertensive animals, if I understand right, you have more α_{1D} adrenoceptors in peripheral arteries. The number of receptors increases without changing the functionality of α_{1D} adrenoceptors.

P. D'Ocon

We really do not know if the number of receptors was increased because we did not measure this parameter. We suppose that the functionality of these receptors was increased. We neither know if the number of receptors located in the membrane and coupled to G proteins is increased.

R. Leurs

Could you explain to me why in the blood vessels of your spontaneously hypertensive animals you see this difference between prazosin and the BMY compound? Both of them were initially presented as inverse agonists, but you only see this change in the pIC_{50} value in one of them.

P. D'Ocon

It is probably due because prazosin has the same affinity for the three subtypes of receptors, and the total activity of the α_{1D} subtypes do not change with the hypertension. There is a change in the proportion between the activity of α_{1D} and the other subtypes. In this case, we only observe the change with BMY 7378, which is the selective ligand for this subtype. I think that in hypertension the total α_1 adrenergic activity does not change.

R. Leurs

Could you speculate on the action of captopril on the α_{1D} activity? It is an intriguing experiment you show, and the hypertensive effect is known, but what's happening with α_{1D} in this respect?

P. D'Ocon

We are really doing the same experiments under chronic and acute treatment with other antihypertensive drugs, such as prazosin, nifedipine and propranolol. We expect to have more results in order to clarify our hypothesis. We do not know if this change is a consequence of the hypertensive state, nor is there a cause.

R. Bond

When Martin Lohse asked you about the affinity of noradrenaline, you probably answered him about potency. Therefore, the affinity is the same for both. In the captopril experiments, presumably nothing has changed other than vascular tone. Regarding your proposed hypothesis, it seems that those experiments would argue against the hypothesis of internalization, since if you just correct tone—from hypertensive to normotensive—the effect goes away. Is that right?

P. D'Ocon

I think there are different situations. In the normal situation, the process of internalisation could occur in all receptors systems. However, we do not know what is the mechanism that mediates this pathological situation in hypertension, if this increase is mediated by internalisation, increasing the number or by any other mechanisms. According to our results, we only know that the functionality of α_{1D} adrenoceptors is increased in hypertension.

R. Bond

I do not mean noradrenaline that is left contaminating, but there is something subthreshold in those vessels. You are saying that the α_{1D} receptor has like a memory. It seems from the captopril experiments that it requires something in the milieu like to make it flip, rather than having to go inside and back outside.

P. D'Ocon

Captopril is just normalizing the situation. The same as do with the α_{1D} and the receptor functionality.

J. Stankova

I was just wondering, now that the tools are available, whether you have any evidence that the α_{1D} adrenoceptor changes his localisation after stimulation, whether it goes from the inside out.

P. D'Ocon

We are now trying to confirm this hypothesis. We are working with Prof. McGrath from Glasgow University in order to determine this possible movement of α_{1D} adrenoceptor with confocal microscopy.

G. Milligan

You can hopefully look at this using a fluorescently labelled form of prazosin. My question is, do you feel that the intracellular α_{1D} adrenoceptor is actually functional, or has to reach the cell surface before it can generate a signal?

P. D'Ocon

I think that the internal receptor could be functional, but at another level, not at G protein level. It could be involved in processes regulating cell growth or other kind of signals into the cell.

The interaction with G proteins in the membrane necessarily needs that the receptors move to the membrane. It is for this that we analyse a high inositol phosphate accumulation, because this signal is directly related to the membrane.

A. IJzerman

I like these studies very much, because they are moving away from genetically engineered cell systems, more into the direction of the clinic. In addition, the pathophysiological evidence that you showed is very nice.

However, in your experiments you apply noradrenaline to the vessel organ bath. Could you do similar experiments in which you allowed the system itself to produce the noradrenaline? That would give us yet another idea about how relevant it really is in the physiology of the animal. So, could you use electrical stimulation such that noradrenaline is being released rather than applied?

P. D'Ocon

The problem is that α_{1D} adrenoceptors have a functional role in vessels poorly innervated and not in vessels densely innervated. Then it is difficult to plan an experiment as you propose.

A. IJzerman

If you think about the real physiological relevance, then how much noradrenaline could be around the α_{1D} adrenoceptors in an intact situation?

P. D'Ocon

I do not know the concentration of noradrenaline or adrenaline. In fact, α_{1D} adrenoceptors are in vessels where probably adrenaline is the most important adrenergic stimulus.

M. Lohse

The circulating concentration of noradrenaline is about a third of the circulating adrenaline, so that should work with either drug.

I would like to come back to the kinetic experiment that you showed. It is still in my mind because in the α_2 adrenoceptors we have similar behaviour: α_{2A} subtype is a fast, and α_{2C} subtype is a slow receptor. You were saying the decay of the response is slow, but I think you also showed that the onset of the response is slow. It seems like this is a receptor, which switches back slowly but it also switches on slowly.

P. D'Ocon

Yes, that is true. However, I cannot give you this data because we did not measure the time until obtain the maximal response.