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MINI REVIEW

Presynaptic autoreceptors in the third decade: focus on α_2-adrenoceptors

Klaus Starke

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Freiburg, Germany

Presynaptic autoreceptors were discovered by six groups of researchers in 1971. The receptors of 1971 were autoreceptors regulating the release of noradrenaline, acetylcholine and GABA (Starke *et al*. 1989).

The first 10 years after 1971 yielded the basic evidence. Carlsson (1975) coined the term 'autoreceptors', receptors 'sensitive to the neuron's own transmitter substance'. It was recognized that neurons may also possess autoreceptors in their soma-dendritic region. The second 10 years brought important pharmacological consequences: just like presynaptic α-autoreceptors had been the first α_2-adrenoceptors (Langer 1974), presynaptic serotonin autoreceptors now became the prototypical 5-HT_1-receptors, presynaptic histamine autoreceptors the prototypical H_3-receptors (Starke *et al*. 1989), and presynaptic glutamate autoreceptors activated by L-2-amino-4-phosphonobutyrate (L-AP4) became the prototypical metabotropic glutamate receptors (Monaghan *et al*. 1989). The second 10 years also witnessed a spirited denial of the function, and even the existence, of autoreceptors (Kalsner 1982; Starke 1987; Kalsner and Westfall 1990), an assault that, I believe, miscarried because the observations spoke, and speak, for themselves.

The purpose of this article is to summarize events of the third decade of autoreceptor research, following our review of 1989 (Starke *et al*. 1989). Novel features comprised the further pharmacological classification of the autoreceptors, their molecular identification, their study by means of receptor-deficient animals and a detailed elucidation of their signal transduction. These research steps of the third decade were common to presynaptic autoreceptors. However, instead of giving a general survey here, which would have to remain superficial, I shall discuss one group of autoreceptors at some depth, the release-inhibiting α_2-autoreceptors of noradrenergic neurones. They were the first in which an inhibitory effect of the transmitter itself was demonstrated (Starke 1972). They are among the best-studied, and their study is representative of the study of other presynaptic autoreceptors. However, attention should be drawn to the fact that presynaptic glutamate autoreceptors, with ionotropic and metabotropic and with inhibitory and facilitatory members, were a focal point of interest in the last 10 years (MacDermott *et al*. 1999).

α_2-Autoreceptors: α_2-adrenoceptor subclassification

Presynaptic α_2-autoreceptors were not only the prototype α_2-adrenoceptors (see above). Their study also showed first that α_2-adrenoceptors were not one single receptor type: they differed between rats and rabbits (Hieble *et al*. 1997). Radioligand binding experiments then indicated that there were four pharmacologically distinct α_2-adrenoceptors, α_{2A}, α_{2B}, α_{2C} and α_{2D} (Bylund *et al*. 1994). On the basis of molecular genetic studies it is now generally accepted that many mammalian species including humans, rats, mice and guinea pigs possess three α_2-adrenoceptor genes, of which one codes for the $\alpha_{2A/D}$-adrenoceptor, one for the α_{2B}-adrenoceptor and one for the α_{2C}-adrenoceptor. The designation $\alpha_{2A/D}$ mirrors the fact that small differences in amino acid sequence lead to notable species differences in the pharmacological properties of this receptor, the receptor with α_{2A} pharmacology (characterized for example by an antagonist affinity ratio phentolamine $<<$ yohimbine, rauwolscine) occurring in humans and rabbits and the orthologous receptor with α_{2D} pharmacology (characterized, for example, by an antagonist affinity ratio phentolamine $\geq$ yohimbine, rauwolscine) in rodents. Part of the pharmacological difference can be attributed to a point mutation in the fifth transmembrane domain of this receptor where cysteine in humans is replaced by serine in mice and rats (Link *et al*. 1992; Bylund *et al*. 1994; Hieble *et al*. 1997; Hein 2001).

α_2-Autoreceptors: subtype determination

With the genetic trichotomy of α_2-adrenoceptors established, the question arose to which subtype or subtypes the presynaptic α_2-autoreceptors belonged. Comparison of antagonist potencies at the autoreceptors with their affinities for prototypical α_{2A}, α_{2B}, α_{2C} and α_{2D} binding sites indicated that presynaptic autoreceptors were at least predominantly α_{2A} in humans and rabbits and α_{2D} in rats and mice, i.e. belonged to the genetic $\alpha_{2A/D}$ branch with its two orthologous varieties, or in other words: mammals seemed to express mainly the $\alpha_{2A/D}$ gene in noradrenergic neurones to bring these neurones under α_2-autoreceptor

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Address and reprint requests to Dr Klaus Starke, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Hermann-Herder-Strasse 5, D-79104 Freiburg i.Br., Germany.

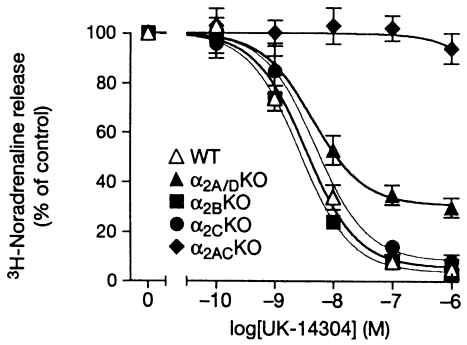

Fig. 1 Inhibition by the α_2-adrenoceptor agonist UK-14304 of electrically evoked [^{3}H]noradrenaline release in atria from wild-type mice (WT) and mice in which either the $\alpha_{2A/D}$-adrenoceptor gene, the α_{2B}-adrenoceptor gene, the α_{2C}-adrenoceptor gene or both the $\alpha_{2A/D}$- and the α_{2C}-adrenoceptor gene had been disrupted. From Hein *et al.* (1999).

control (Trendelenburg *et al*. 1993; Limberger *et al*. 1995; Guimarães *et al*. 1998; Feuerstein *et al*. 2000; Docherty 1998). The identification was unambiguous for cerebral noradrenergic neurones. It was less clear-cut for post-ganglionic sympathetic neurones. The autoreceptors of the sympathetic fibres of the human kidney and human atria, for example, were initially classified as α_{2C} (Trendelenburg *et al*. 1994a; Rump *et al*. 1995; but also see Trendelenburg *et al*. 1997), and the autoreceptors of rat and mouse atria also did not show pure α_{2D} characteristics (Limberger *et al*. 1992; Wahl *et al*. 1996).

The generation of mice with disruptions of the $\alpha_{2A/D}$, α_{2B} or α_{2C} gene ($\alpha_{2A/D}$KO, α_{2B}KO, α_{2C}KO) offered a novel approach to the problem. These studies have confirmed the suggestion that the main mammalian presynaptic α_2-autoreceptors are $\alpha_{2A/D}$ (Altman *et al*. 1999; Hein *et al*. 1999; Trendelenburg *et al*. 1999, 2001a, 2001b). For example, the maximal inhibition of noradrenaline release produced by the selective α_2-adrenoceptor agonist medetomidine in the hippocampus and occipito-parietal cortex was reduced from almost total in wild-type preparations to about 37% in $\alpha_{2A/D}$KO preparations, and in atria and the vas deferens it was reduced from almost total (wild-type) to about 77% ($\alpha_{2A/D}$KO; Trendelenburg *et al*. 1999). In contrast, the maximal inhibition of noradrenaline release produced by medetomidine was not changed in the hippocampus, the occipito-parietal cortex, atria and the vas deferens of α_{2B}KO or α_{2C}KO mice (Altman *et al*. 1999; Trendelenburg *et al*. 2001b). However, some inhibition by medetomidine remained in all $\alpha_{2A/D}$KO tissues. Interestingly, this remaining, non$\alpha_{2A/D}$-adrenoceptor-mediated inhibition was greater (~ 77% maximally) in atria and the vas deferens than in the hippocampus and the occipito-parietal cortex (~ 37% maximally), as mentioned, in accord with the notion from studies with series of antagonists that it was in peripheral tissues that the pharmacology of the autoreceptors deviated from pure α_{2A} or α_{2D} characteristics. The nature of the non$\alpha_{2A/D}$-autoreceptor in mouse atria was clarified when Hein *et al*. (1999) generated mice that lacked both the $\alpha_{2A/D}$- and the α_{2C}-adrenoceptor (α_{2AC}KO). Figure 1 shows the effect of the α_2 agonist UK-14304 on the electrically evoked release of noradrenaline in atria taken from wild-type and the various KO mice. The maximal inhibition obtained in wild-type atria was near 100%, and this was not changed when the tissues lacked the α_{2B}- or α_{2C}-adrenoceptor. In atria lacking the $\alpha_{2A/D}$-adrenoceptor, the maximal inhibition was reduced to 70% (Fig. 1). When the α_{2C}-adrenoceptor had been deleted in addition to the $\alpha_{2A/D}$-adrenoceptor to produce α_{2AC}KO animals, however, this remaining inhibition was lost and UK-14304 failed to produce any effect (Fig. 1). The non-$\alpha_{2A/D}$-autoreceptors in mouse atria hence were α_{2C}. The same was true for the mouse brain cortex, except that here the non-$\alpha_{2A/D}$ component was smaller (Hein *et al*. 1999). So when one extrapolates beyond the species and tissues tested, mammals possess two presynaptic α_2-autoreceptors, $\alpha_{2A/D}$-autoreceptors which predominate, and α_{2C}-autoreceptors, which are more prominent in peripheral tissues than in the brain. The failure of deletion of the α_{2C}-adrenoceptor alone to produce any change indicates that, as long as $\alpha_{2A/D}$-adrenoceptors are present they suffice for full autoreceptor function, at least as examined by means of an exogenous agonist (however, see section on physiological operation below).

The α_2-autoreceptor subtype has also been examined in post-ganglionic sympathetic neurones from newborn mice kept in primary culture. As shown in Fig. 2(a), UK 14,304 greatly reduced the electrically evoked release of noradrenaline from wild-type cultures. Phentolamine was a more potent antagonist against UK 14,304 than rauwolscine, giving the typical α_{2D} antagonist potency ratio phentolamine > rauwolscine. In accord with this diagnosis, no inhibition whatsoever remained when the $\alpha_{2A/D}$ gene had been disrupted (Fig. 2b): the presynaptic autoreceptors were purely $\alpha_{2A/D}$ (i.e. pharmacologically α_{2D}). No α_{2C} admixture as in adult mouse sympathetically innervated tissues was found, perhaps because the expression of α_{2C}-autoreceptors begins only a couple of days after birth (see section on development below).

The soma-dendritic α_2-autoreceptors of noradrenergic neurones mediate an increase in potassium conductance, a decrease in calcium currents and therefore inhibition. Their subtype has been investigated both in the locus coeruleus and in sympathetic ganglion cells. Studies with antagonists showed that, in the intact rat locus coeruleus, the main soma-dendritic α_2-autoreceptors mediating hyperpolarization and a decrease of firing were $\alpha_{2A/D}$ (Nörenberg *et al*. 1997; Mateo and Meana 1999). However, an induction of inwardly rectifying potassium currents in acutely dissociated

rat locus coeruleus neurones was mediated by α_{2B}- or α_{2C}-adrenoceptors (Arima *et al*. 1998). In the cultured mouse sympathetic neurones mentioned above, the major part of the inhibition of calcium currents caused by UK 14,304 was mediated by $\alpha_{2A/D}$-adrenoceptors. However, some inhibition remained in neurones taken from $\alpha_{2A/D}$KO mice, indicating a component of α_{2B}- or α_{2C}-adrenoceptors (Trendelenburg *et al*. 2001a). So when one extrapolates again, in accord with presynaptic α_2-autoreceptors mammals seem to possess two soma-dendritic α_2-autoreceptors, $\alpha_{2A/D}$-autoreceptors which predominate, and a minor population of either α_{2B}- or α_{2C}-autoreceptors.

The question of differences between α_2-autoreceptors and other α_2-adrenoceptors has interested many researchers, inter alia because of possible therapeutic consequences. So-called α_2-heteroreceptors occur on cerebral serotoninergic terminal axons where they inhibit the release of serotonin. Studies with series of antagonists indicated that, in the brain of rats and rabbits, the presynaptic α_2-autoreceptors and the heteroreceptors at the serotoninergic axons were similar, both being mainly α_{2A} in rabbits and α_{2D} in rats (Trendelenburg *et al*. 1994b). This conclusion has now also been confirmed in genetically manipulated mice. Like the α_2-autoreceptors, the heteroreceptors in the hippocampus were a mixture of predominant $\alpha_{2A/D}$- and minor α_{2C}-adrenoceptors. The serotonin release-inhibiting effect of the agonist medetomidine was reduced in $\alpha_{2A/D}$KO and α_{2C}KO tissues and disappeared completely when both receptors had been eliminated, i.e. in α_{2AC}KO tissues (Scheibner *et al*. 2001a).

α_2-Autoreceptors: in search for the molecules

For many years researchers have tried to label α_2-autoreceptors by radioligands, with limited success (Starke 1987; Starke *et al*. 1989). The cloning of the three α_2-adrenoceptor genes opened up new possibilities.

One major result has been the demonstration that noradrenergic neurones express the $\alpha_{2A/D}$-adrenoceptor gene as studied by *in situ* hybridization of the mRNA. This has been shown both in noradrenergic brain nuclei, especially the locus coeruleus, of rats (Nicholas *et al*. 1993; Scheinin *et al*. 1994; Winzer-Serhan *et al*. 1997a) and mice (Wang *et al*. 1996) and in the rat superior cervical ganglion (Vidovic *et al*. 1994). In fact, 'the most intense hybridization signal in the entire rat brain was seen with the α_{2A} probe in the locus coeruleus', and the authors suggest that 'this receptor subtype functions as a presynatic autoreceptor in the rat brain' (Scheinin *et al*. 1994). α_{2B}- and α_{2C}-adrenoceptor mRNA was not detected in noradrenergic

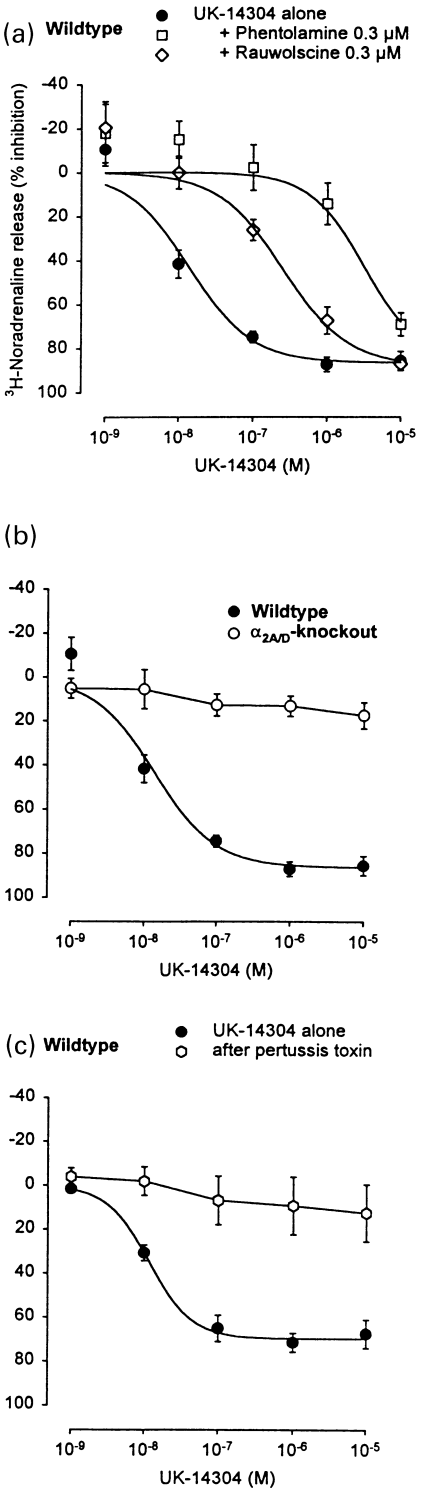

Fig. 2 Inhibition by the α_2-adrenoceptor agonist UK-14304 of electrically evoked release of [^{3}H]noradrenaline in cultures of postganglionic sympathetic neurones taken from newborn wild-type or $\alpha_{2A/D}$-adrenoceptor-deficient mice. (a) Interaction of UK-14304 with antagonists in neurones from wild-type mice. (b) Comparison of neurones from wild-type and $\alpha_{2A/D}$KO mice. (c) Effect of pretreatment of wild-type cultures with pertussis toxin. From Trendelenburg *et al.* (2001a).

cells or was detected only in very small amounts (Vidovic et al. 1994; Winzer-Serhan *et al*. 1997b).

A second major result was the immunological demonstration of both the $\alpha_{2A/D}$- and the α_{2C}-adrenoceptor protein in cerebral noradrenergic nuclei, in particular the locus coeruleus (Rosin *et al*. 1993, 1996). The proteins were located at least partly in the membrane of the noradrenergic neurone dendrites (Lee *et al*. 1998a,b).

So both the mRNA and the protein studies agree well with the functional studies: the molecules functioning as α_2-autoreceptors, i.e. mainly $\alpha_{2A/D}$- and to a minor extent α_{2C}-adrenoceptors, are in fact synthesized, and present, in noradrenergic neurones. However, some gaps in this correspondence of function and protein presence remain. Although cerebral soma-dendritic α_2-autoreceptors have been demonstrated immunologically as proteins in the appropriate dendritic membranes, an analogous demonstration of an axon terminal, i.e. presynaptic, α_2-autoreceptor protein in the brain has not yet been achieved. Moreover, α_2-adrenoceptor proteins have not been demonstrated on peripheral post-ganglionic sympathetic neurones.

The latter gap may soon be filled. Olli-Lähdesmäki *et al*. (1999) transfected rat phaeochromocytoma (PC12) cells, which have many similarities with post-ganglionic sympathetic neurones, with the human $\alpha_{2A/D}$-, α_{2B}- and α_{2C}-adrenoceptor genes and then visualized the receptors with immunocytochemistry. When differentiation and axon growth were induced by nerve growth factor, the α_{2B}-adrenoceptors were evenly distributed over the plasma membrane. The α_{2C}-adrenoceptors were found mainly intracellularly. The $\alpha_{2A/D}$-adrenoceptors, however, were targeted to the membrane of growth cones and later seen in the distal segments of the axons: the very places where they could inhibit neurotransmitter release.

α_2-Autoreceptors: signal transduction

Soon after the discovery of presynaptic α_2-autoreceptors, the idea emerged that they modified calcium movements in noradrenergic terminal axons (Starke and Montel 1974; Göthert 1977; see Starke 1987; Starke *et al*. 1989). With the discovery that all α_2-adrenoceptors couple to heterotrimeric G-proteins, the first signal transduction step was also identified. Much of the presynaptic α_2-adrenergic inhibition is blocked by pertussis toxin (Fig. 2c), but some inhibition may also operate through pertussis toxin-insensitive G-proteins (Allgaier *et al*. 1985; Boehm *et al*. 1992; Hill *et al*. 1993; Koh and Hille 1997; Schwartz 1997). The α_2-adrenergic inhibition of noradrenaline release from chick cultured sympathetic neurones operated only as long as release was due to calcium entry through N-type channels, suggesting that presynaptic N-type calcium channels were the only target of the α_2-autoreceptor-G-protein signal transduction pathway (Boehm and Huck 1996).

The conclusions so far were drawn from noradrenaline release studies. More details have been suggested by studies on soma-dendritic α_2-autoreceptors, most frequently in the rat superior cervical ganglion, because only the soma-dendritic receptor mechanisms are accessible to electrophysiological measurements. These studies have led to the following hypothesis. In rat superior cervical sympathetic ganglion cells, agonist activation of soma-dendritic α_2-autoreceptors leads primarily to activation of G_o or G_i, i.e. dissociation of G_o into $G_{o\alpha}$ and the corresponding $\beta\gamma$-complex and dissociation of G_i into $G_{i\alpha}$ and the corresponding $\beta\gamma$-complex. The $\beta\gamma$-complexes then are more important than the G_α subunits in continuing signal transduction. The $\beta\gamma$-complexes interact directly, without intercalation of a cytoplasmic second messenger, with the pore-forming α_1 subunits of N- and P/Q-type calcium channels, thus reducing the channels' open probability. The α_2-adrenoceptor-G_o pathway seems to predominate; it is pertussis toxin-sensitive, rapid and voltage-dependent: depolarization alleviates the block of the calcium channel. The α_2-adrenoceptor-G_i pathway is pertussis toxin-resistant, slow and voltage-insensitive (Delmas *et al*. 1999; Kaneko *et al*. 1999). The specific $G_{o\alpha}$ proteins in rat superior cervical ganglion cells seem to be $G_{o\alpha A}$ and $G_{o\alpha B}$, and the specific $G_{i\alpha}$ protein seems to be $G_{i\alpha 2}$ (Jeong and Ikeda 2000). Whether the same α_2-adrenoceptor subtype couples to both G_o and G_i, or whether different subtypes couple to each G-protein selectively, is not known. In cultured mouse post-ganglionic sympathetic neurones, pertussis toxin blocked both the $\alpha_{2A/D}$-adrenoceptor-mediated and the non-$\alpha_{2A/D}$-adrenoceptor mediated inhibition of calcium currents (Trendelenburg *et al*. 2001a).

However fascinating this elaborate hypothesis is, it should be remembered that it is based on observations on soma-dendritic receptors. If applicable to presynaptic autoreceptors, it might explain for example why the inhibition of noradrenaline release through α_2-autoreceptors (as well as presynaptic heteroreceptors such as cannabinoid receptors) declines at high frequencies (Göbel *et al*. 2000): high frequency depolarization might remove the voltage-sensitive part of the $\beta\gamma$ blockade of N-type calcium channels. However, some observations on presynaptic α_2-adrenergic inhibition are hard to understand on the basis of any inhibition of calcium entry through voltage-sensitive channels. Other possible modes of inhibition such as direct interference with the exocytotic machinery should be kept in mind (Jackisch *et al*. 1992; Schwartz 1997; Smith and Cunnane 1998).

α_2-Autoreceptors: physiological operation

The question of the physiologial operation of autoreceptors is whether, apart from being targets of exogenous agonists, the autoreceptors are also activated by previously released transmitters itself. The question was the core issue of the

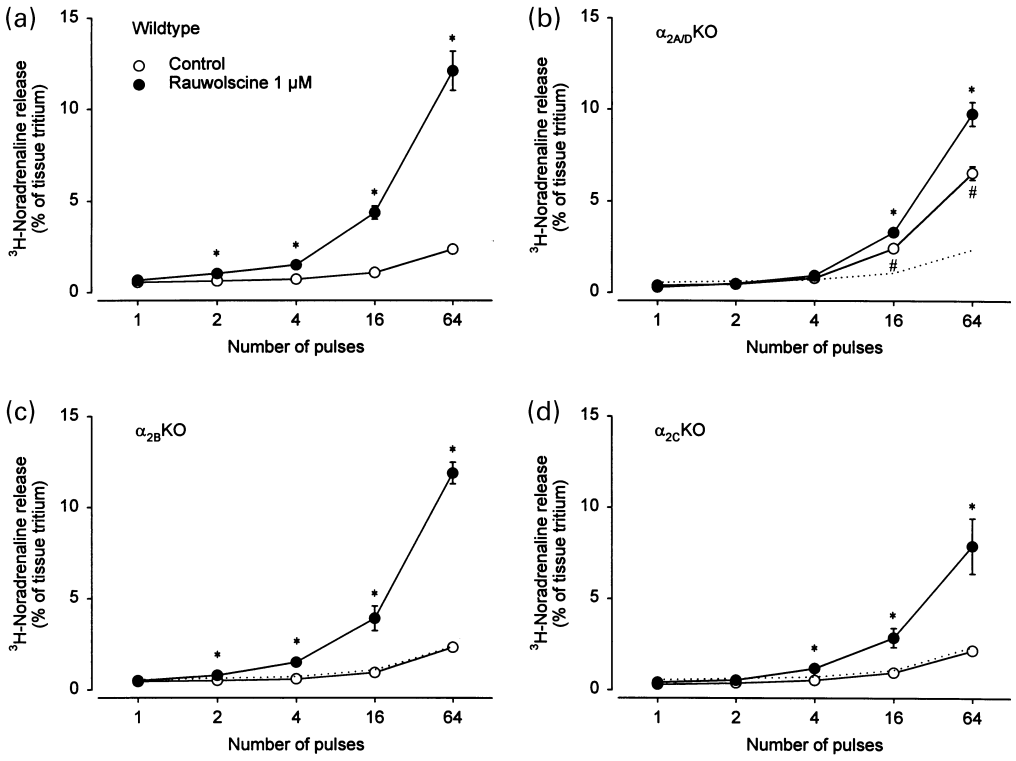

Fig. 3 Pulse number–[^{3}H]noradrenaline release relationships in the brain cortex of wild-type mice and mice in which either the $\alpha_{2A/D}$-adrenoceptor gene, the α_{2B}-adrenoceptor gene or the α_{2C}-adrenoceptor gene had been disrupted. Frequency of stimulation was 1 Hz. Tissues were superfused either with rauwolscine-free medium or with medium containing 1 μM rauwolscine. Dotted lines in b to d represent wild-type release in the absence of rauwolscine (from a). Significant differences from wild-type, no rauwolscine: #$p < 0.05$. Significant effects of rauwolscine: *$p < 0.05$. Modified from Trendelenburg *et al.* (2001b).

struggle, mentioned in the Introduction, that arose in the second decade of autoreceptor research. The most important observation to support an endogenous presynaptic α_2 autoinhibition is the uniform release-enhancing effect of α_2-adrenoceptor antagonists (Starke *et al.* 1989). Again, mice in which α_2-adrenoceptor genes are disrupted offer a new test.

In initial studies, deletion of the $\alpha_{2A/D}$-adrenoceptor increased the release of noradrenaline from mouse atria, vas deferens, occipito-parietal cortex and hippocampus (Hein *et al.* 1999; Trendelenburg *et al.* 1999). In atria, deletion of the α_{2C}-adrenoceptor also increased the release of noradrenaline, and release was increased even more when both receptors were deleted to yield α_{2AC}KO atria (Hein *et al.* 1999). These investigations confirmed the classical work with α_2-adrenoceptor antagonists and showed that the autoreceptors indeed mediated a physiological negative feedback.

More recently, effects of both gene disruptions and the α_2 antagonist rauwolscine have been examined in several mouse tissues over a wide range of stimulation pulse numbers and stmulation frequencies (Scheibner *et al.* 2001b; Trendelenburg *et al.* 2001b). Figure 3 shows the pulse number-noradrenaline release relationship in the brain cortex of wild-type, $\alpha_{2A/D}$KO, α_{2B}KO and α_{2C}KO mice, the frequency being constant (1 Hz). In wild-type brain cortex in the absence of rauwolscine, the pulse number-release curve was flat, release by p pulses being much lower than p times the release by a single pulse, and rauwolscine did not increase the release elicited by a single pulse but increased greatly the release elicited by 2 or more pulses, thus rendering the curve much steeper (Fig. 3a). In α_{2B}KO and α_{2C}KO brain cortex there was no change from wild-type (Figs 3c and d). Only deletion of the $\alpha_{2A/D}$-adrenoceptor changed the pattern; in the $\alpha_{2A/D}$KO brain cortex, the pulse number-release curve was steepened, release by p pulses being much closer to p times the release by a single pulse, and rauwolscine increased the release of noradrenaline but slightly (Fig. 3b). Essentially similar results were obtained in the hippocampus, in atria and in the vas deferens. So in all tissues, α_2-autoinhibition greatly depressed (and its removal by rauwolscine or $\alpha_{2A/D}$ gene disruption hence greatly increased) the slope of the pulse number-release relationship. The non-additivity of the effects of $\alpha_{2A/D}$ gene disruption and rauwolscine clearly showed that the two interventions acted by the

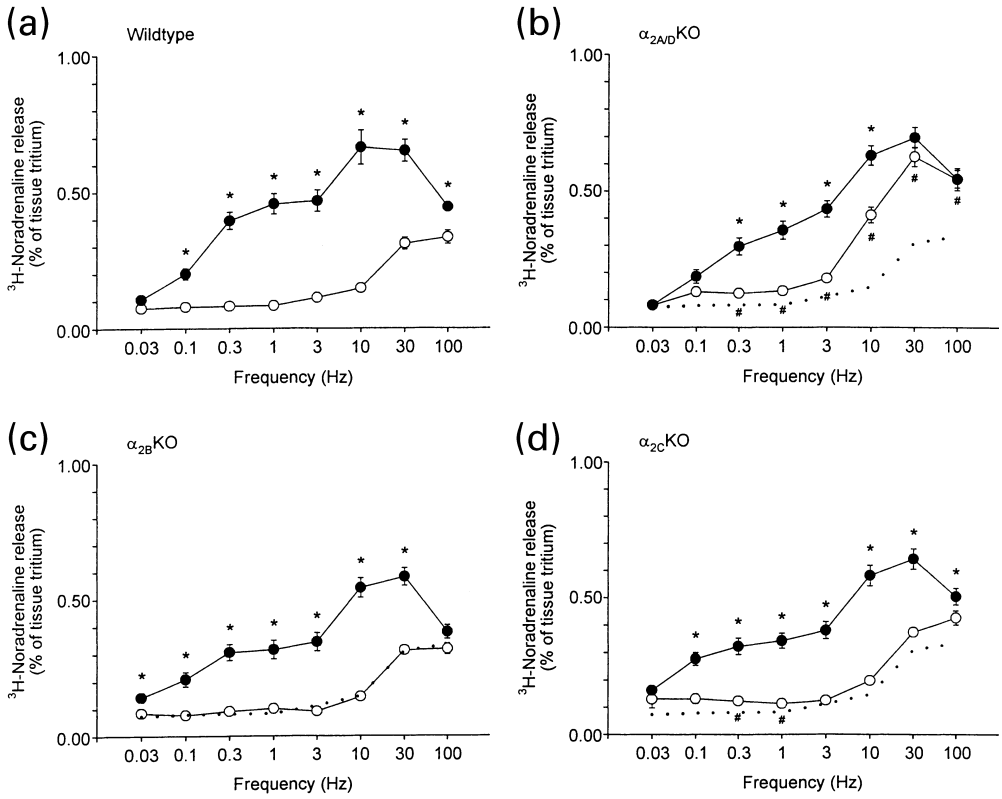

Fig. 4 Frequency–[^{3}H]noradrenaline release relationships in the vas deferens of wild-type mice and mice in which either the $\alpha_{2A/D}$-adrenoceptor gene, the α_{2B}-adrenoceptor gene or the α_{2C}-adrenoceptor gene had been disrupted. Trains of 30 pulses were applied for each frequency. Tissues were superfused either with rauwolscine-free medium or with medium containing 1 μM rauwolscine. Dotted lines in b to d represent wild-type release in the absence of rauwolscine (from a). Significant differences from wild-type, no rauwolscine: #$p < 0.05$. Significant effects of rauwolscine: *$p < 0.05$. From Scheibner *et al.* (2001b).

same mechanism, i.e. interruption of α_2-autoinhibition. In these experiments, the only unambiguous autoreceptor was $\alpha_{2A/D}$ (but see below).

Figure 4 shows the frequency-noradrenaline release relationship in the vas deferens of wild-type, $\alpha_{2A/D}$KO, α_{2B}KO and α_{2C}KO mice, the pulse number being constant (30). In wild-type vas deferens in the absence of rauwolscine, the curve increased monophasically with frequency, and rauwolscine caused large increases, except at the lowest frequency of 0.03 Hz, and slightly steepened the curve (Fig. 4a). Nothing changed in the α_{2B}KO vas deferens (Fig. 4c). In α_{2C}KO vas deferens, the release elicited by 0.3- and 1- Hz trains was slightly higher compared to wild-type, and rauwolscine still caused large increases (Fig. 4d). Deletion of the $\alpha_{2A/D}$-adrenoceptor changed the pattern to a greater extent; in the $\alpha_{2A/D}$KO vas deferens, release of noradrenaline was considerably increased as compared to wild-type over a wide range of frequencies, the curve was steepened, and rauwolscine increased release only slightly (Fig. 4b). So in the vas deferens, α_2 autoinhibition depressed (and its removal by rauwolscine or $\alpha_{2A/D}$ gene disruption hence increased) the slope of the frequency-release relationship. The non-additivity of the effects of $\alpha_{2A/D}$ gene disruption and rauwolscine again showed that both shared a common mechanism, i.e. interruption of α_2-autoinhibition. Although the $\alpha_{2A/D}$-adrenoceptor was the main autoreceptor in these experiments, α_{2C}-autoreceptors also became manifest in a twofold manner: as the increase in release caused by α_{2C} gene disruption (Fig. 4d), and as the increase by rauwolscine that remained in the $\alpha_{2A/D}$KO vas deferens (Fig. 4b).

The classical effects of the α_2 antagonist and the new observations on α_2-adrenoceptor gene-deficient mice concur to demonstrate that presynaptic α_2-autoreceptors are physiological sites of action of released noradrenaline. A presynaptic negative feedback indeed operates. It helps to shape fundamental properties of transmitter release, namely the pulse number-noradrenaline release relationship and the frequency-noradrenaline release relationship. Both autoreceptors contribute to this physiological function. Possibly α_{2C}-autoreceptors inhibit mainly release at low frequencies (see Fig. 4d) and $\alpha_{2A/D}$-autoreceptors inhibit mainly release at high frequencies (Fig. 4b).

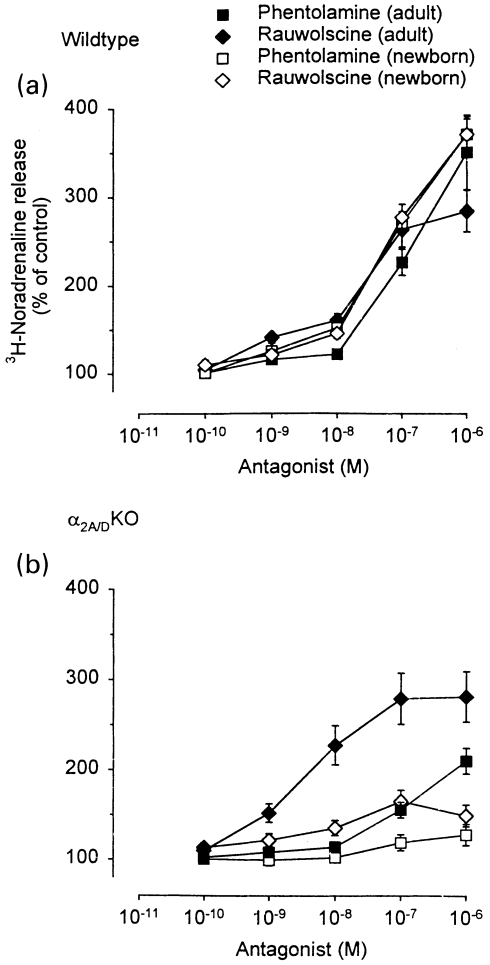

Fig. 5 Enhancement by phentolamine and rauwolscine of [^{3}H]noradrenaline release in atria from wild-type mice and mice in which the $\alpha_{2A/D}$-adrenoceptor gene had been disrupted. Atria were taken either from newborn or from adult mice. From Schelb *et al.* (2001).

α_2-Autoreceptors: postnatal development

Presynaptic α_2-autoreceptors operate already at birth, both in the brain (Wemer and Mulder 1981) and in the periphery (Guimarães *et al*. 1994). The question now to be raised is whether $\alpha_{2A/D}$- and α_{2C}-autoreceptors agree or differ in their development. Recent evidence suggest that they differ, at least in the peripheral tissues where the α_{2C}-receptors contribute notably to autoinhibition. In support of this view, Fig. 5 shows α_2-autoinhibition in atria of newborn and adult wild-type and $\alpha_{2A/D}$KO mice, the degree of autoinhibition being indicated by the noradrenaline release-enhancing effect of the antagonists phentolamine and rauwolscine. In wild-type atria, the antagonists greatly increased the release of noradrenaline, irrespective of whether the hearts were taken from newborn or adult animals (Fig. 5a). In $\alpha_{2A/D}$KO atria, in contrast, the antagonists caused marked increases only when the hearts were taken from adults (Fig. 5b). α_{2C}-Autoinhibition is the inhibition (revealed by the antagonist-induced increases) remaining in $\alpha_{2A/D}$KO tissues. It is minimal in atria of newborn mice and much more marked in adults (Fig. 5b). $\alpha_{2A/D}$-Autoinhibition is the difference in inhibition (revealed by the antagonist-induced increases) between wild-type (Fig. 5a) and $\alpha_{2A/D}$KO tissues (Fig. 5b). It is large in atria of newborn mice and becomes less dominant in adults. So the first α_2-autoreceptors to appear in atria are $\alpha_{2A/D}$; they mediate the (already substantial) α_2-autoinhibition in wild-type atria at birth and do not gain much in efficiency later. α_{2C}-Autoreceptors, in contrast, seem to be expressed, or at least to become functional, only during postnatal development. A similar differential development was found in the vas deferens (Schelb *et al*. 2001).

Outlook

Research on presynaptic autoreceptors in general and α_2-autoreceptors in particular has matured in its third decade. Greeted with some surprise in the early seventies, the autoreceptors are now established elements of the regulation of neurones. As to the next decade of research, one may wish to see presynaptic α_2-autoreceptors visualized as beautifully in the membrane of noradrenergic terminal axons as certain metabotropic glutamate autoreceptors in the membrane of glutamatergic axons (Takumi *et al*. 1999). One may also wish to see the presynaptic signal transduction elucidated as completely, step by step, as the signal transduction following activation of soma-dendritic α_2-autoreceptors. Lastly, the relative tasks of $\alpha_{2A/D}$- and α_{2C}-autoreceptors will have to be discovered, and one will have to see whether, contrary to expectation, α_{2B}-adrenoceptors also may serve as autoreceptors under certain conditions, a possibility for which there is some, but scant, evidence (Scheibner *et al*. 2001b).

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