ELSEVIER

European Journal of Pharmacology 295 (1996) 275–280

ejp

Short communication

Apparent desensitization of a σ receptor sub-population in neonatal rat cardiac myocytes by pre-treatment with σ receptor ligands

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Received 27 October 1995; accepted 3 November 1995

Abstract

σ Receptor ligands induce marked effects on contractility in cardiac myocytes from neonatal and adult rats (Ela et al., 1994, J. Pharmacol. Exp. Ther. 269, 1300–1309; Novakova et al., 1995, Eur. J. Pharmacol. 286, 19–30). Augmentation or attenuation of the contractile amplitude was observed under different experimental conditions. Preincubation of neonatal cardiomyocytes with a σ receptor ligand ((+)-(3-hydroxyphenyl)-*N*-(1-propyl)-piperidine ((+)-3PPP), (+)-pentazocine, or haloperidol) changed the response to re-application of the ligand after cell wash. The inhibitory effect was abolished, while the stimulatory effect became much more pronounced. We suggest that the effects of σ receptor ligands are mediated via two receptor subtypes, one stimulatory and the other inhibitory, and only the inhibitory subtype is subject to desensitization.

Keywords: σ Receptor; Cardiac myocyte; Contractility

1. Introduction

σ Receptors are present in the central nervous system and in many peripheral organs, but neither their functional role nor their mechanism of action is fully understood. Results of several studies have led to the suggestion that σ receptors may have antipsychotic, antiemetic, antitussive and neuroprotective effects, and may be involved in regulation of posture and movement. In vitro studies in neuronal preparations have shown that σ receptor ligands inhibit agonist stimulated phosphoinositide turnover, reduce NMDA enhanced cGMP level, and cause blockade of tonic K^+ channels. In the periphery, σ receptors appear to potentiate electrically stimulated contraction of rat and mouse vas deferens, as well as contraction of muscle/myenteric plexus preparations from guinea-pig. Several other regulatory functions in endocrine, immune and gastrointestinal systems have been ascribed to σ receptors but the mode of action of these receptors is still undetermined (see recent reviews in Itzhak, 1994).

Much of the confusion regarding the function of σ receptors is caused by the existence of multiple types of σ receptors (Quirion et al., 1992), and by the fact that most of the effects are induced by concentrations of ligands much higher than the K_d values obtained in binding studies. Controversial results in several systems are also a cause of uncertainty. Opposite effects of different σ receptor ligands, or of the same ligand at different concentrations have been reported. Examples of such effects are the potentiation (Kennedy and Henderson, 1989) and inhibition (De Haven-Hudkins et al., 1991) of contraction of mouse vas deferens and of guinea-pig muscle/myenteric plexus (Coccini et al., 1991), concentration dependent enhancement or inhibition of NMDA-stimulated noradrenaline release from rat hippocampal slices (Monnet et al., 1992), and opposite effects of different σ receptor ligands on dopaminergic activity (reviewed by Walker et al., 1994).

Recently we have reported that σ receptor ligands modulate contractility and Ca^{2+} transients in cardiac myocytes from neonatal and adult rats (Ela et al., 1994; Novakova et al., 1995). Cardiac myocytes from neonatal rat responded to nanomolar concentrations of σ

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[1] This study is a part of a Ph.D. thesis to be submitted by Ms C. Ela to the senate of the Hebrew University.

SSDI 0014-2999(95)00750-4

receptor ligands by a typical pattern of changes in the contractile amplitude. The amplitude of contraction decreases by 10–25% 1–2 min after drug application, then transiently increases (3–10 min) and finally decreases to about 75% of the control level (Ela et al., 1994). Exposure of isolated cardiomyocytes from adult rats to σ receptor ligands induces a different response. (+)-3PPP, haloperidol or (+)-pentazocine (10 nM each) caused a substantial increase in contractile amplitude in the majority (75%) of the examined cells. Approximately 25% of the examined cells did not show any increase in the amplitude of contraction but a decrease to about 70–80% of control. Exposure of the cardiomyocytes to lower concentrations of haloperidol or (+)-pentazocine (1 nM) induced a decrease in the amplitude of contraction in all examined cells (Novakova et al., 1995).

Analysis of the patterns of response to σ receptor ligands in neonatal and adult rat cardiomyocytes suggests that the effects on contractility are mediated by two sub-populations or two conformations of the high affinity σ_1 receptors, one mediating an increase and the other a decrease in contractile amplitude.

In the present study we investigated this suggestion. After pre-treating neonatal rat cardiomyocytes with a σ receptor ligand followed by an extensive cell wash, the response to a second application of the ligand was different from that to the first one. The results are consistent with a model suggesting desensitization of one of the two sub-populations of σ receptors.

2. Materials and methods

Cultures of ventricular myocytes from neonatal rats were prepared as described previously (Ela et al., 1994). After trypsinization of the ventricular fragments of 1-day-old Sabra rat hearts, and pre-plating the cell suspension to remove fibroblasts, the cardiac myocytes were plated on circular coverslips (25 mm) which were placed in Petri dishes. The cultures were maintained in humidified 5% CO_2-95% air atmosphere at 37°C for 4 days before the experiments.

Contractility was measured as described previously (Ela et al., 1994). The glass coverslips with attached cardiac myocytes were placed in a temperature controlled cell chamber, equipped with a glass bottom, and were superfused constantly with balanced salt solution containing (millimolar): NaCl, 140; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; glucose, 10; Na_2HPO_4, 1; and Hepes buffer, 10, pH 7.4. The cardiac myocytes were field stimulated via two platinum electrodes placed in the superfusion solution. The amplitude of contraction was measured by recording the movement of a microsphere attached to the surface of the cultured cells, by using a phase-contrast microscope–video motion detector system, as previously described (Ela et al., 1994).

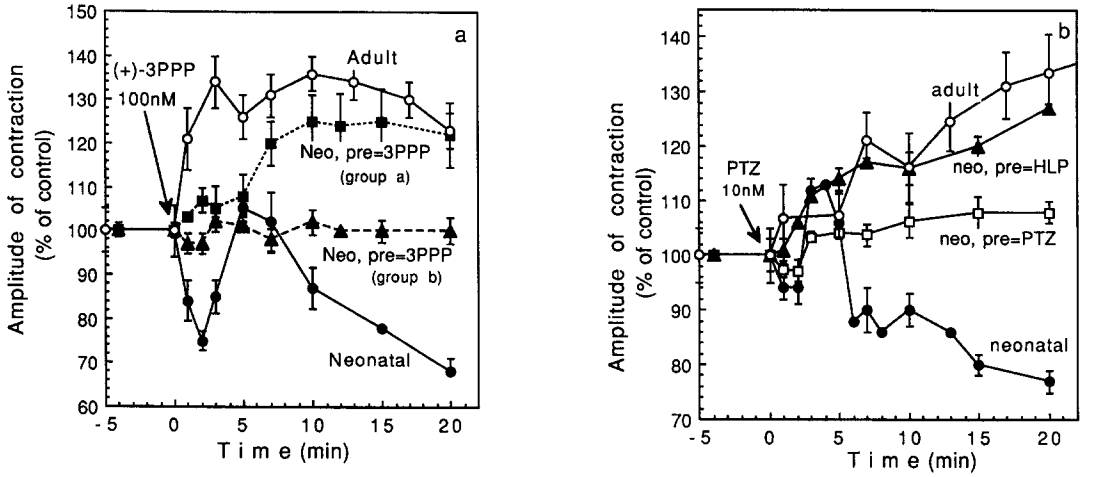

Fig. 1. Effects of prolonged incubation of cultured neonatal rat cardiac myocytes with σ receptor ligands on the subsequent response to re-exposure to a homologous or heterologous σ receptor ligand. Cultures of neonatal rat cardiomyocytes were incubated overnight with (a) 100 nM (+)-3PPP (pre = 3-PPP) or (b) 100 nM (+)-pentazocine (pre = PTZ) or 100 nM haloperidol (pre = HLP). Each drug was added to the growth medium. Before the experiment, the medium was removed and the cultures were washed for 90 min with balanced salt solution without the σ receptor ligand; during this period the solution was changed 8 times. In every experiment, control cultures from the same preparation were treated similarly but without σ receptor ligand (neonatal). The amplitude of contraction was then continuously recorded before and after the re-addition of (a) 100 nM (+)-3PPP or (b) 10 nM (+)-pentazocine (PTZ). Results are expressed as percentages of the amplitude of contraction determined in the same cells before the re-addition of the σ receptor ligand as indicated. In (a), results of 12 experiments on cultures preincubated with (+)-3PPP were grouped according to two distinct patterns of response observed after the re-exposure to the drug. In group a, we calculated the means (±S.E.M.) of results obtained in 8 of 12 cultures which showed a marked increase in the amplitude of contraction. In group b, we calculated the means (±S.E.M.) of results obtained in 4 of 12 cultures in which there was no change in the amplitude of contraction. For comparison, effects of (a) 100 nM (+)-3PPP or (b) 10 nM haloperidol on the amplitude of contraction in isolated cardiac myocytes from adult rats are presented (adult).

Isolated cardiac myocytes from the ventricles of adult rat hearts were prepared and the contractility was measured as described previously (Novakova et al., 1995).

Results are expressed as the percentage of the contractile amplitude of the same cell before drug addition. Means ± S.E.M. of several experiments (as indicated) are presented.

3. Results

Our previous results in cardiomyocytes from neonatal and adult rats led to the suggestion that the effects of σ receptor ligands on the contractile amplitude are mediated via two sub-populations or two conformations of the high affinity σ_1 receptors. We tried therefore to differentiate between the effects mediated by the postulated σ receptor subtypes. A procedure which proved to be successful was subjecting the neonatal cardiomyocytes to desensitization of σ receptors by preincubating the cultures overnight with σ receptor ligands.

In the first group of experiments, cultures were preincubated overnight with 100 nM (+)-3PPP. Following this preincubation, the cultures were washed 8 times, for a total of 90 min, with balanced salt solution without (+)-3PPP and then (+)-3PPP (100 nM) was applied again. The amplitude of contraction was determined before and after the second exposure to (+)-3PPP in cultures preincubated with (+)-3PPP and in control cultures from the same preparation which were subjected to a similar washing procedure. The mean value of the contractile amplitude in cultures preincubated overnight with (+)-3PPP was 0.83 ± 0.1 μm (mean ± S.E.M., $n = 12$), whereas in the control cultures the mean value was 0.91 ± 0.12 μm (mean ± S.E.M., $n = 3$). The difference between these values is not significant.

The control cultures responded to (+)-3PPP by a typical pattern of changes in the amplitude of contraction as previously observed (decrease–increase–decrease) (Fig. 1a, neonatal). Cardiac myocytes which were preincubated overnight with (+)-3PPP displayed two different response patterns to re-application of (+)-3PPP. In 8 of 12 experiments, an increase in the amplitude of contraction, reaching 125 ± 6% (mean ± S.E.M., $n = 8$) of the control level was observed. There was no initial decrease in the contractile amplitude as seen in control cultures (Fig. 1a, neo, group a). In these cultures, the pattern of response to (+)-3PPP became remarkably similar to that of adult cells (Fig. 1a, adult). In 4 of 12 experiments, the second exposure to (+)-3PPP did not cause any change in the amplitude of contraction (Fig. 1a, neo, group b).

Similar results were obtained after pre-treatment with 100 nM (+)-pentazocine and exposure to 10 nM (+)-pentazocine. Cultured cardiac myocytes from neonatal rats were preincubated overnight in medium containing 100 nM (+)-pentazocine and then washed extensively during 90 min with balanced salt solution. Control cultures from the same preparation which were not exposed to (+)-pentazocine were washed similarly. After the wash (+)-pentazocine (10 nM) was applied to the preincubated and control cultures. The control cultures responded with a typical pattern of decrease–increase–decrease in contractile amplitude (Fig. 1b, neonatal), whereas most of the cultures which had been preincubated with (+)-pentazocine (5 out of 6) showed a small increase in the amplitude of contraction (108 ± 1.8% of control level, mean ± S.E.M., $n = 5$) in response to exposure to 10 nM (+)-pentazocine (Fig. 1b, neo, pre = PTZ). Similar experiments were conducted with cultures preincubated overnight with 100 nM haloperidol. After the preincubation and an extensive cell wash, 10 nM (+)-pentazocine was applied. In these cultures exposure to (+)-pentazocine

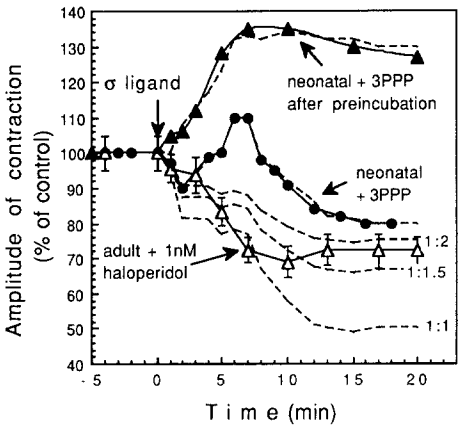

Fig. 2. Predictions of a model assuming that the effects of σ receptor ligands are mediated by two receptor subtypes, one inhibitory and one stimulatory. This model is based on the assumption that the magnitude of the changes in the amplitude of contraction in neonatal cardiomyocytes exposed to a σ receptor ligand is the sum of the changes induced by activation of two receptor subtypes, one mediating a decrease and the other an increase in the contractile amplitude. Preincubation leads to desensitization of the inhibitory subtype. The theoretical contribution of the inhibitory subtype to the changes in the amplitude of contraction in neonatal cardiomyocytes was calculated from the responses of neonatal cardiac myocytes to 100 nM (+)-3PPP without and after preincubation. The calculation was based on the simplified assumption that the preincubation did not affect the stimulatory subtype but completely abolished the contribution of the inhibitory subtype. The simulated curves (broken lines) are based on ratios between the contributions of stimulatory and inhibitory subtypes of 1:1, 1:1.5 and 1:2. For comparison, a curve showing the results obtained in adult rat cardiomyocytes exposed to 1 nM haloperidol (under which condition only the inhibitory effect is expressed) is presented. This simulation is intended to show the feasibility of the model. By no means should it be considered as an attempt to present a proof.

caused an increase in the contractile amplitude to 127 ± 3.6% of the control level (mean ± S.E.M., $n = 7$) in all 7 examined cultures (Fig. 1b, neo, pre = HLP). The pattern of response of neonatal cardiac myocytes to σ receptor ligands after preincubation with a σ receptor ligand became similar to that of adult cardiac myocytes (Fig. 1b, adult).

Fig. 2 shows the predictions of a model which assumes that the magnitude of the changes in the amplitude of contraction in neonatal cardiac myocytes exposed to a σ receptor ligand is the sum of the changes induced by activation of two receptor subtypes: σ_1^{dec}, mediating a decrease and σ_1^{inc}, mediating an increase in the amplitude of contraction. Preincubation leads to desensitization of σ_1^{dec}. (In a few cultures both subtypes appear to be desensitized.) The theoretical contribution of σ_1^{dec} to the σ receptor ligand-induced changes in the contractile amplitude of neonatal cardiac myocytes was calculated from the experimental results (responses of neonatal cardiac myocytes to (+)-3PPP without preincubation and after preincubation). The calculation was based on the simplified assumption that the preincubation did not affect σ_1^{inc} but completely abolished σ_1^{dec}. The putative contribution of σ_1^{dec} was calculated assuming ratios between the contributions of σ_1^{inc} to σ_1^{dec} of 1:1, 1:1.5 and 1:2. The calculated curves are similar to results obtained in adult cardiac myocytes exposed to 1 nM haloperidol. This concentration of haloperidol induced a decrease in the amplitude of contraction in adult rat cardiac myocytes (Novakova et al., 1995).

4. Discussion

Our group has previously shown the presence of authentic σ binding sites on cardiac myocytes from neonatal and adult rats. In these cells nanomolar concentrations of prototypic σ receptor ligands exerted effects on contractility, Ca^{2+} transients and Ca^{2+} fluxes (Ela et al., 1994; Novakova et al., 1995). Neonatal rat cardiomyocytes exhibited changes in contractility which were dependent on the time of exposure to the σ receptor ligand. An initial decrease in the contractile amplitude was followed by a transient increase and a final decrease. This response could be interpreted as due to two simultaneous actions, potentiation and attenuation of the amplitude of contraction, each mediated by a distinct sub-population of receptors. A different time course of response for each of the receptor sub-populations would yield the observed pattern. Obviously, other interpretations may be suggested, such as conformational change in the receptors or changes in the signal transduction pathways which mediate the response (Ela et al., 1994).

Cardiac myocytes from adult rats displayed different responses. Exposure to 1 nM (+)-pentazocine or haloperidol induced a decrease in contractile amplitudes. Exposure to 10 nM (+)-pentazocine, haloperidol or (+)-3PPP caused an increase in contractile amplitude in about 75% of the cells while the rest of the cells displayed a decrease in amplitude. Higher concentrations of (+)-3PPP caused only an increase in the amplitude of contraction. The apparent ED_{50} value for the maximal increase in contractile amplitude by (+)-3PPP was 4.5 nM. This value is only slightly lower than the K_d value determined by binding experiments in cardiomyocyte membranes from adult rats (Novakova et al., 1995).

(+)-Pentazocine and (+)-3PPP are highly specific ligands for σ receptors at concentrations of 10 nM (Bowen et al., 1993; Hellewell et al., 1994). Haloperidol also binds to dopamine receptors. However, the similarity between the effects exerted by nanomolar concentrations of haloperidol and of the prototypic σ receptor ligands (+)-pentazocine and (+)-3PPP in cardiac myocytes from neonatal and adult rats may indicate that the effects of haloperidol are also mediated by σ receptors (Ela et al., 1994; Novakova et al., 1995).

In the present study we continued to investigate the effects of the same prototypic σ receptor ligands. It was found that exposure of neonatal cardiac myocytes to prolonged incubation with one of the σ receptor ligands, (+)-3PPP, haloperidol or (+)-pentazocine (100 nM), followed by an extensive cell wash, affected the response to a second application of a homologous σ receptor ligand ((+)-3PPP or (+)-pentazocine, 10 nM) or heterologous σ receptor ligand ((+)-pentazocine, 10 nM, after preincubation with haloperidol, 100 nM). The pattern of response of neonatal cardiac myocytes to re-exposure to a σ receptor ligand became similar to the response of adult rat cardiomyocytes, i.e. an increase in the amplitude of contraction was observed. Neither the preincubation alone nor the cell wash alone affected the contractile amplitude.

These results may suggest the presence of two receptor subtypes, one mediating augmentation and the other attenuation of the contractile amplitude. We propose that prolonged preincubation with a σ receptor ligand led to functional desensitization of the receptor subtype which mediates the attenuation of the amplitude of contraction. It is not clear why the other postulated subtype, which mediates the augmentation of the contractile amplitude, is not subject to desensitization. In adult cardiac myocytes, 10 min exposure to a σ receptor ligand, followed by a short cell wash, prevented an increase in contractile amplitude in response to re-application of the ligand. These results were interpreted as due to desensitization of the receptor subtype which mediates the augmentation of the amplitude of contraction (Novakova et al., 1995). The ab-

sence of desensitization of this putative receptor subtype in neonatal cardiac myocytes is difficult to explain. A plausible suggestion is the occurrence of re-sensitization of this receptor subtype during the prolong cell wash. However, our results do not support this hypothesis. When the cell wash was shortened to 15 min, the pattern of response to re-application of the ligand was similar to that obtained after prolonged cell wash (data not shown).

An alternative model is the presence of two conformations of the same receptor subtype, one mediating augmentation and the other attenuation of the amplitude of contraction. According to this model, the response of neonatal cardiac myocytes to σ receptor ligands would be mediated by the two forms of the receptor. Preincubation with a σ receptor ligand would cause a shift toward the conformation mediating the augmentation of contractile amplitude. Adult cardiac myocytes appear to display predominantly the conformation mediating the augmentation of the amplitude of contraction, but under specific conditions (very low ligand concentration or exposure to σ receptor ligand after preincubation with the ligand), the conformation mediating the attenuation of the contractile amplitude would prevail. We do not have enough information, at present, to decide between the two models.

Receptor desensitisation is usually mediated by receptor down-regulation which can be shown by radioligand binding experiments. However, in this case binding experiments may lead to biased conclusions. A decrease in the number of binding sites after preincubation with a σ receptor agonist may indicate either receptor down-regulation, or incomplete wash-out of the preincubating ligand. These two mechanisms could be distinguished by application of an antagonist with similar affinity as the agonist to the receptors. Preincubation with the antagonist would not cause receptor down-regulation, therefore a decrease in the number of binding sites after preincubation would indicate incomplete ligand wash-out. Unfortunately, such an antagonist is not available.

The existence of the high affinity σ_1 and the low affinity σ_2 receptor subtypes is now well established (Quirion et al., 1992). However, in the present work both postulated receptor subtypes appear to be related to the high affinity σ_1 receptor since the effects are exerted by very low concentrations of (+)-pentazocine which is selective for σ_1 receptors (Bowen et al., 1993). Several recent studies in different tissues suggest the presence of σ receptor subtypes in addition to the subtypes classified as 1 and 2. For example, in guinea-pig brains, 1,3-di-*o*-tolylguanidine (DTG) binds to at least three σ binding sites (Connick et al., 1992). A new subtype of σ receptors in rat brain has recently been suggested on the basis of binding and functional studies (Booth et al., 1993). In guinea-pig and rat brain membranes, (+)-3PPP and DTG bind to pharmacologically distinct σ sites (Karbon et al., 1991).

Down-regulation/desensitization of σ receptors has been previously demonstrated in vivo. Repeated injections of haloperidol to rats led to down-regulation of σ receptors and to reduced motor response to intra-nigral and intra-rubral microinjection of (+)-pentazocine (Matsumoto et al., 1989).

Conformational changes in σ receptors have also been suggested, mainly on the basis of radioligand binding: (+)-pentazocine binding to guinea-pig cerebellum is modulated by divalent cations (Basile et al., 1992). In adrenal chromaffin cells nicotine allosterically modulates the binding of (+)-pentazocine to σ receptors (Paul et al., 1993). Polyamines inhibited allosterically (+)-3PPP binding to σ receptors (Paul et al., 1990).

An overview of the information on functions mediated by σ receptors leads to the suggestion that these receptors modify the action of other effectors rather than mediate effects of their own. In cardiac myocytes, σ receptor ligands modify electrically evoked contractile amplitudes with no effect on resting cells. The physiological role of σ receptors may therefore be mainly the regulation of the magnitude of evoked responses in a diverse array of systems according to an unknown set of signals. We suggest that this regulation may lead to either potentiation or attenuation of the target functions, by selective activation of one of two different receptor subtypes or conformations, each mediating an opposite effect.

Some conflicting results in the literature have led to uncertainty as to whether σ receptor ligands potentiate or inhibit certain functions. In several cases different ligands acted in different directions causing confusion as to which are agonists and which are antagonists (or rather 'reversed agonists'). Some examples are the effect of iontophoretically applied σ receptor ligands on firing in rubral neurons. Whereas dextrallorphan and DTG induced inhibition, (+)-3PPP induced potentiation of neuronal firing (Matsumoto and Walker, 1992). In guinea-pig muscle/myenteric plexus, DTG, haloperidol and (+)-3PPP inhibited while (+)-SKF-10047 augmented contraction (Coccini et al., 1991). Potentiation of rat and guinea-pig vas deferens contraction by σ receptor ligands has been reported by several laboratories and inhibition of contraction has been reported by others (review by Su and Junien, 1994). In other studies the same ligand exerted opposite effects. Similar to our results in adult cardiac myocytes (Novakova et al., 1995), NMDA-induced release of noradrenaline was potentiated by low concentrations of σ receptor ligands and inhibited by higher concentrations of the same ligands in rat hippocampal slices (Monnet et al., 1992).

The suggested model may offer an explanation for

results showing opposite effects of σ receptor ligands on various systems. We are, however, aware that there are no solid data to support this model. It should therefore be viewed merely as a working hypothesis and a guideline for further research.

Acknowledgements

This research was supported by a grant from the Chief Scientist of the Ministry of Health, Israel (to Y.E.).

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