Short communication

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Somatic and dendritic perforated-patch recordings reveal β-adrenergic receptor-induced depolarization in medial prefrontal cortex pyramidal neurons

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The aim of this perforated-patch study was to test the effect of isoproterenol on the membrane potential in mPFC (medial prefrontal cortex) pyramidal neurons. Isoproterenol depolarized the membrane potential recorded from the soma. This effect was absent in the presence of metoprolol, suggesting the involvement of β1-adrenergic receptors. The adenylate cyclase activator forskolin also depolarized the membrane potential. Moreover, the effect of isoproterenol was abolished by the adenylate cyclase inhibitor SQ 22536. This suggested that adenylate cyclase was involved in mediating the effect of the β-adrenergic receptor agonist. The isoproterenol-induced depolarization persisted after inhibition of protein kinase A with H-89. The effect of β-adrenergic receptor activation on the membrane potential was dependent on I_h channels because it was abolished in the presence of the I_h channel inhibitor ZD 7288. Dendritic recordings were also performed. In the dendritic segments between 100 μm and 150 μm from the soma and between 200 μm and 250 μm from the soma, isoproterenol also depolarized the membrane potential. The magnitude of the β-adrenergic receptor-stimulated depolarization was the same in the soma and in both dendritic localizations. The depolarization exerted by isoproterenol may influence PFC cognitive functions.

Key words: medial prefrontal cortex, β-adrenergic receptors, membrane potential, dendrite, isoproterenol, perforated-patch

The PFC (prefrontal cortex) is involved in the pathophysiology of many psychiatric diseases, for example, PTSD (post-traumatic stress disorder), depression and schizophrenia. Working memory dysfunction, poor concentration and impulsivity are characteristic symptoms of PFC dysfunction (Hains and Arnsten 2008, Ramos and Arnsten 2007). The onset of psychiatric disease may be precipitated by stress, which is associated with the excessive release of noradrenaline (Ramos and Arnsten 2007).

There are three types of β-adrenergic receptors (β1, β2 and β3). They are located in the peripheral organs, as well as the brain, for example, in the amygdala, hippocampus and prefrontal cortex. The brain's β-adrenergic receptors are activated by noradrenaline, which is endogenously released from the locus coeruleus in the brainstem (Hains and Arnsten 2008, Ramos and Arnsten 2007).

β-adrenergic receptors are metabotropic receptors, and their activation modifies the electrophysiological properties of neurons through a signal transduction cascade (Pisani et al. 2003, Szulczyk 2015). For example, somatic recordings showed that isoproterenol enhances sodium currents in mPFC pyramidal neurons (Szulczyk 2015) and calcium currents in the amygdala (Huang et al. 1998). It has also been shown in hippocampal neurons in whole-cell configuration that isoproterenol increases the amplitude of dendritic action potentials (Yuan et al. 2002).

The aim of this study was to test the effect of β-adrenergic receptor activation on the membrane potential in the soma and in the dendrites of mPFC pyramidal neurons. Somatic perforated-patch and extremely rare dendritic perforated-patch recordings are presented.

The experimental procedures used in this study adhered to the institutional and international guidelines for the ethical use of animals. The experiments were performed on the neurons of 3-week-old male rats. After the induction of deep anaesthesia using ethyl chloride, the brains were removed and placed in a cold (0–4°C) solution that contained the following compounds (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), $MgCl_2$ (7) and $CaCl_2$ (0.5), at a pH of 7.4 and at an osmolality of 320 mOsm/kg H_2O. Coronal PFC slices (280 μm thick) were cut in the same solution. The apical dendrites of the pyramidal neurons were parallel to the slice surface because the slicing stage was angled at 10°. This made the dendrites easier to visualize. After cutting, the slices

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were incubated for 30 minutes under warm temperature (34°C) in standard ACSF (artificial cerebrospinal fluid), which contained the following compounds (in mM): NaCl (130), KCl (2.5), glucose (10), $NaHCO_3$ (25), NaH_2PO_4 (1.25), $MgCl_2$ (2) and $CaCl_2$ (2), at a pH of 7.4 with carbogen and an osmolality of 320 mOsm/kg H_2O.

The recordings were performed in the same solution at a temperature of 35°C. Images of neurons were recorded using DIC optics. The recordings were obtained from layer V pyramidal neurons in the infralimbic and prelimbic mPFC, at a depth of 600–800 μm from the cortical surface.

To visualize the apical dendrites of PFC pyramidal neurons, the slices should be cut at an angle in high magnesium solution (see above). In my previous study (Szulczyk 2015), the dendrites of the PFC pyramidal neurons were difficult to visualize, presumably because the PFC slices were not cut at an angle, and a sucrose-based solution was used to cut the slices.

The membrane potentials were recorded under current-clamp conditions in gramicidin perforated-patch recording mode (Szulczyk 2015, Szulczyk et al. 2012). The pipette solution contained the following compounds (in mM): potassium gluconate (105), KCl (20), HEPES-Na^+ (10), and ethylene glycol-bis-(2-aminoethylether)-N,N,N′,N′--tetraacetic acid (0.1), at a pH of 7.25 and an osmolality of 280 mOsm/kg H_2O. The pipette solution also included gramicidin (80–100 microgram/ml for somatic recordings and 160–200 microgram/ml for dendritic recordings). The pipettes' resistances were 6–7 megaOhms and 17–20 megaOhms for the somatic and dendritic recordings, respectively.

After obtaining a tight seal, progress during membrane perforation was monitored by observing the slow decrease in access resistance. After the access resistance reached a steady level, a control membrane potential was recorded for a few minutes, and if it remained stable, the influence of isoproterenol was assessed. When the patch ruptured spontaneously, the recording was discarded. The chemical compounds were delivered to the whole bath.

All of the results presented in this paper are shown as the mean ±S.E.M. Differences between more than two groups were evaluated using ANOVA (Analysis of Variance), followed by Tukey's post hoc test (GraphPad InStat software v3.06). Student's t-test was also used.

All recordings were conducted in the presence of 1 μM TTX (Pisani et al. 2003) to block action potential-dependent synaptic transmission (with the exception of Fig. 1Bb and 3Db). The membrane potential was −65.2±2.2 mV in the control, −62.0±2.2 mV after isoproterenol (2 μM) application and −64.0±2.6 mV after washout (n=6, $F_{3,17}$=13.1, P<0.001, Fig. 1Aab). Thus, ß-adrenergic receptor activation depolarized the membrane potential of mPFC pyramidal neurons by 3.2±0.2 mV (n=6, Fig. 1Aab).

The finding that isoproterenol depolarizes the membrane potential in the soma of mPFC pyramidal neurons is also included in my previous study, which is about the influence of isoproterenol on sodium channels (Szulczyk 2015). In the previous study, however, all recordings were conducted at room temperature, which is in contrast to the recordings in this study, which were made at 35°C. Moreover, in the previous study (Szulczyk 2015), the depolarization caused by isoproterenol was smaller than the one described here, which suggests that the effect of isoproterenol on the membrane potential is temperature-dependent.

To obtain the dose-response relationship, the following doses of isoproterenol were used: 0.02 μM, 0.2 μM, 2 μM and 200 μM, and the average membrane potential changes were 0.1±0.26 mV (n=3, P>0.05), 1.9±0.3 mV (n=4, P>0.01), 3.2±0.2 mV (n=6, $F_{3,17}$=13.1, P<0.001) and 3.0±0.5 mV (n=7, $F_{3,20}$=22.9, P<0.001), respectively (Fig. 1Ba). These results indicate that 2 μM isoproterenol exerts the maximal effect on the membrane potential. Thus, this dose was used throughout the study.

In the absence of TTX in the extracellular solution, isoproterenol (2 μM) depolarized the membrane potential by 3.5±0.3 mV (n=6, Fig. 1Bb, $F_{3,17}$=79.3, P<0.001), the same as with TTX in the bath (see above, P>0.05, Fig. 1Bb). These experiments show that action potential-dependent synaptic transmission does not contribute to the effect of isoproterenol on the membrane potential.

In the constant presence of the β1-adrenergic receptor antagonist metoprolol (60 mM) in the bath, isoproterenol did not depolarize the membrane potential (the average change was 0.6±0.2 mV, n=4, P>0.05, Fig. 1Cab). This finding shows that the effect of isoproterenol depends on a type of β1-adrenergic receptor.

Thereafter, recordings were made in the presence of the I_h channel inhibitor ZD 7288 (30 μM) in the bath. When the I_h channels were blocked, β-adrenergic receptor activation did not influence the membrane potential (the average change was −0.5±0.6 mV, P>0.05, Fig. 1Dab, n=4). It is therefore concluded that isoproterenol exerts its effect via I_h channels.

Moreover, the membrane potential in the presence of the I_h channel inhibitor ZD 7288 (30 μM) in the bath (−74.9±1.3 mV, n=6) was significantly more hyperpolarized than the control membrane potential (−65.2±2.2 mV, n=6, t_8=3.7, P<0.01). This finding is consistent with I_h channel inhibition by ZD 7288.

The adenylate cyclase activator forskolin (30 μM) also depolarized the membrane potential in mPFC pyramidal neurons. The effect increased after washout. The average membrane potential changes were 2.3±0.2 mV after five minutes of forskolin application and 3.4±0.4 mV after a 10 minute wash-out (n=6, Fig. 2Aab, $F_{3,17}$=70.7, P<0.001). These results indicate that forskolin mimicked the effect of isoproterenol on the membrane potential. One may hypothesize that isoproterenol depolarizes the membrane potential via adenylate cyclase activation.

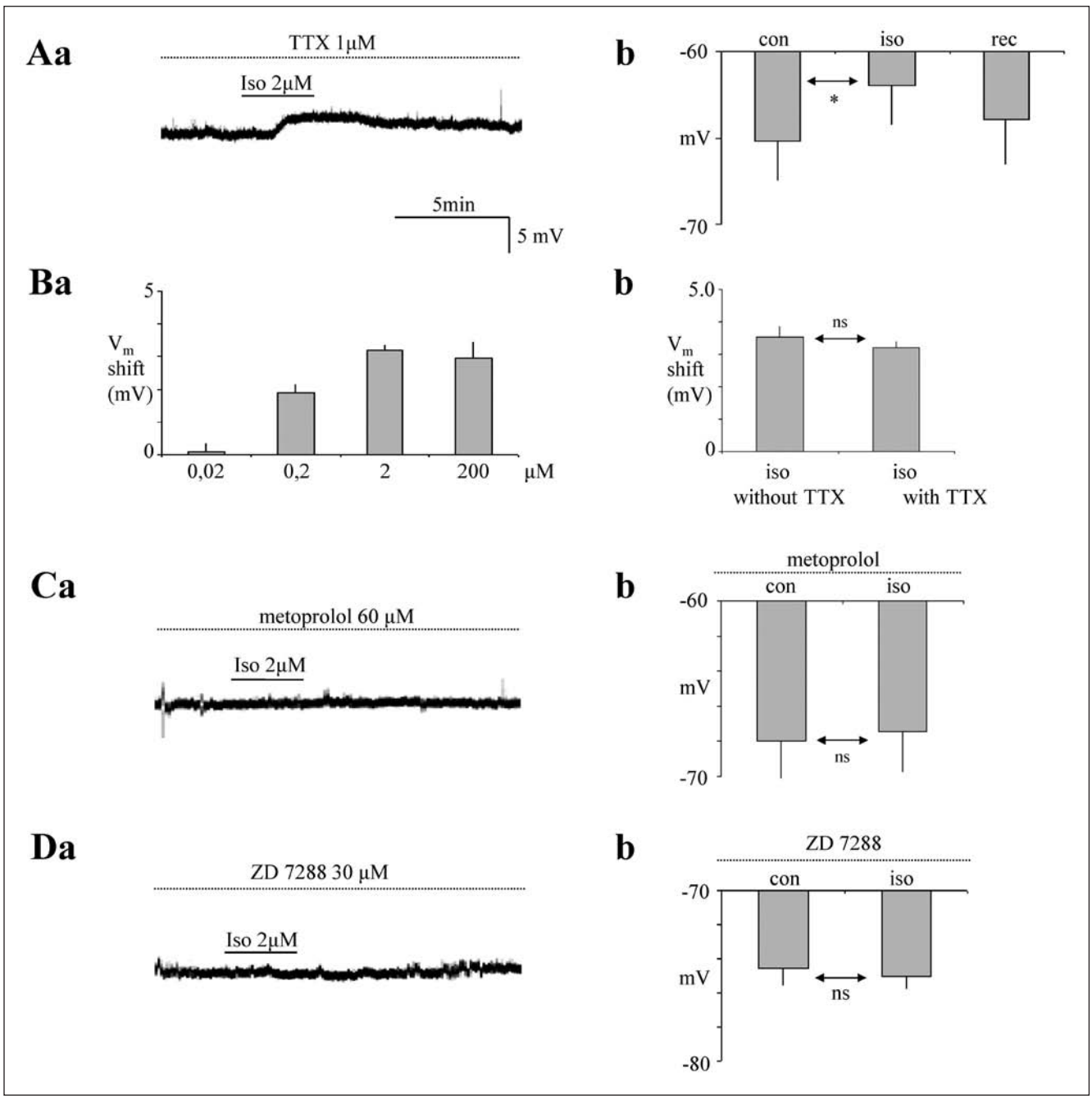

Fig. 1. Isoproterenol depolarizes the membrane potential in mPFC pyramidal neurons via I_h channels (somatic recordings). (A) Isoproterenol depolarizes the membrane potential (Aa – example recording, Ab – averaged membrane potential values). (Ba) Membrane potential changes exerted by different doses of isoproterenol. (Bb) TTX does not influence isoproterenol-induced depolarization. (C) Isoproterenol does not influence the membrane potential in the presence of the ß1-adrenergic receptor antagonist metoprolol (Ca – example recording, Cb – membrane potential values). (D) In the presence of the I_h channel inhibitor ZD 7288, isoproterenol does not depolarize the membrane potential (Da – example recording, Db – membrane potential values). Abbreviations: con., iso. and rec., mean: control, isoproterenol and recovery, respectively; ns means non-significant. Statistical significance is indicated by an asterisk.

To prove this hypothesis, experiments were performed with the adenylate cyclase blocker SQ 22536 (150 μM) in the bath. Under this condition, isoproterenol did not depolarize the membrane potential (the average membrane potential change was 0.8±0.4 mV, Fig. 2Bab, P>0.05, n=5). These experiments prove that isoproterenol exerts its effect via adenylate cyclase.

Adenylate cyclase produces cAMP, which often exerts its effects via protein kinase A activation (Ji et al. 2008). It is also possible, however, that cAMP directly influences

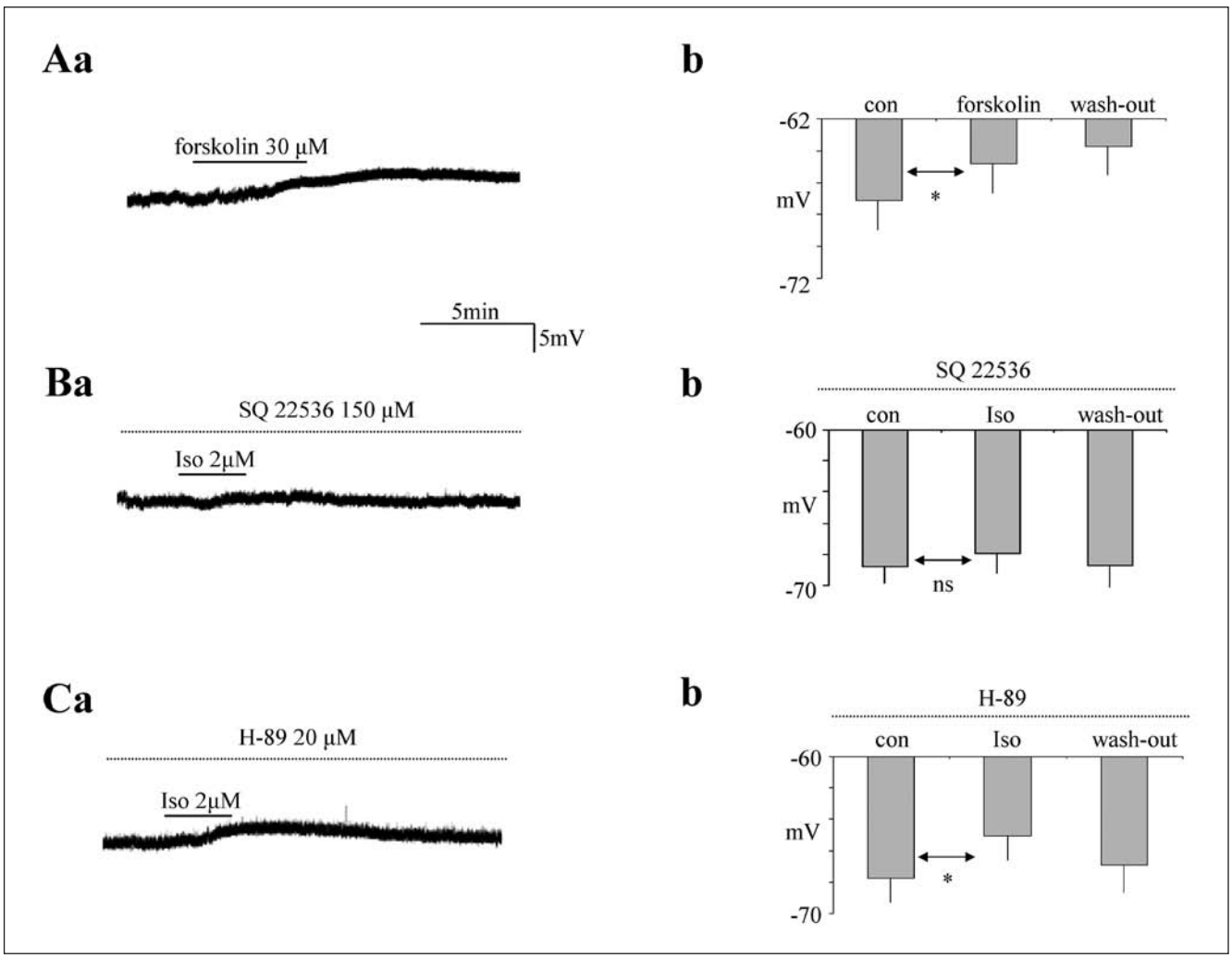

Fig. 2. Signal transduction pathway from the ß1-adrenergic receptor to I_h channels (somatic recordings). (A) The adenylate cyclase activator forskolin depolarizes the membrane potential in mPFC pyramidal neurons (Aa – example recording, Ab – membrane potential values). (B) With the adenylate cyclase inhibitor SQ 22536 in the bath, isoproterenol does not influence the membrane potential (Ba – example recording, Bb – membrane potential values). (C) Preincubation of the slices with the protein kinase A inhibitor H-89 does not change the isoproterenol induced-depolarization (Ca – example recording, Cb – membrane potential values).

Abbreviations: con., iso. and rec., mean: control, isoproterenol and recovery, respectively; ns means non-significant.

Statistical significance is indicated by an asterisk.

cellular effectors (Pisani et al. 2003). To distinguish between these two possibilities, the influence of isoproterenol on the membrane potential was assessed after preincubation of the slices with the protein kinase A inhibitor H-89 (20 µM). Under this condition, the membrane potential change was 2.7±0.3 mV (n=7, $F_{3,20}$=67.1, P<0.001, Fig. 2Cab), the same as without the kinase A inhibitor (3.2±0.2 mV, see above, P>0.05). This suggests that protein kinase A is not involved in the effect of isoproterenol on the membrane potential.

The membrane potential was also recorded from the apical dendrites of mPFC pyramidal neurons in two locations: between 100 µm and 150 µm from the soma (113.2±3.0 µm, n=17) and between 200 µm and 250 µm from the soma (238.3±7.9 µm, n=6). The patch pipette attached to the apical dendrite is shown in Fig. 3A.

In the dendritic segment between 100 µm and 150 µm from the soma, isoproterenol depolarized the membrane potential by 3.6±0.6 mV (n=9, $F_{3,26}$=18.9, P<0.001, Fig. 3Bab). The membrane potentials were –66.5±2.3 mV in the control, –62.6±1.9 mV after isoproterenol application and –64.5±2.4 mV after wash-out (n=9, $F_{3,26}$=18.9, P<0.001, Fig. 3Bab). In the absence of TTX in the bath (all other dendritic recordings were conducted with 1 µM TTX in the bath), the average membrane potential change caused by β-adrenergic receptor activation was 4.1±0.7 mV (n=7, $F_{3,20}$=23.3, P<0.001), similar to recordings with TTX in the bath (see above, P>0.05, Fig. 3Db). Thus, action

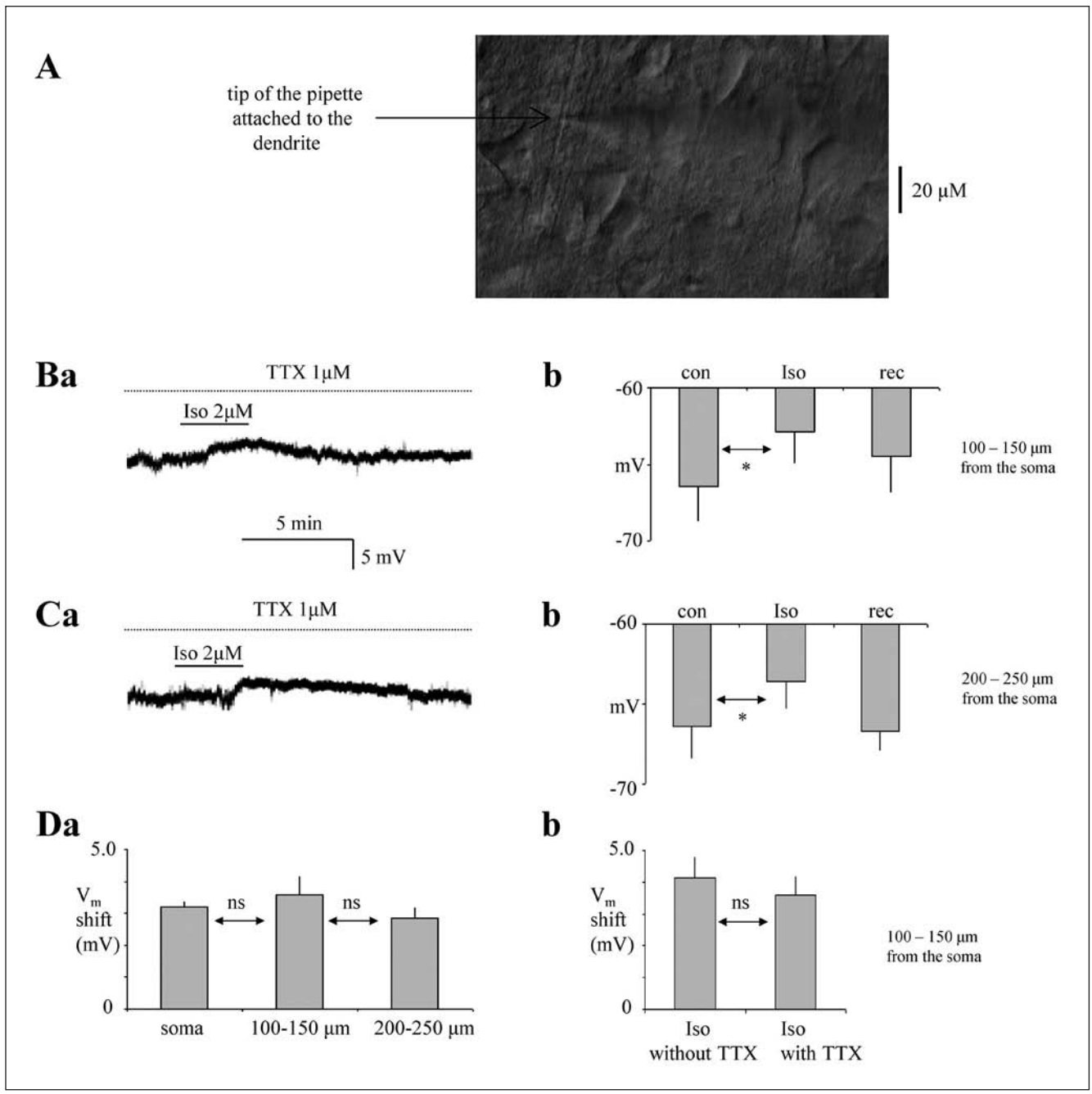

Fig. 3. Isoproterenol depolarizes the membrane potential in mPFC pyramidal neurons (dendritic recordings). (A) Patch pipette attached to the apical dendrite (a DIC image). (B) Isoproterenol depolarizes the membrane potential in the apical dendrite (recordings between 100 mm and 150 mm from the soma, Ba – example recording, Bb – membrane potential values). (C) Isoproterenol depolarizes the membrane potential in a more distal part of the apical dendrite (recordings between 200 mm and 250 mm from the soma, Ca – example recording, Cb – membrane potential values). (Da) The average membrane potential changes exerted by isoproterenol are similar in the soma and in the dendrite. (Db) TTX does not influence isoproterenol-induced depolarization in the dendrite. Abbreviations: con., iso. and rec., mean: control, isoproterenol and recovery, respectively; ns means non-significant.

Statistical significance is indicated by an asterisk.

potential-dependent synaptic transmission did not influence isoproterenol-induced depolarization in the apical dendrites of mPFC pyramidal neurons.

In the dendritic segment between 200 µm and 250 µm from the soma, β-adrenergic receptor activation also depolarized the membrane potential by 2.8±0.4 mV (n=6, $F_{3,17}$=23.7, P<0.001, Fig. 3Cab). The membrane potentials were −66.4±2.0 mV, −63.6±1.7 mV and −66.7±1.2 mV in the control, in the presence of isoproterenol and after wash-out, respectively (n=6, $F_{3,17}$=23.7, P<0.001, Fig. 3Cab).

The control membrane potentials in the soma and in both dendritic locations were not significantly different (P>0.05, see above).

Moreover, the average membrane potential changes exerted by isoproterenol in the soma (3.2±0.2 mV), in the first dendritic segment (3.6±0.6 mV) and in the second dendritic segment (2.8±0.4 mV) were not significantly different (see above, Fig. 3Da, P>0.05).

Perforated-patch recordings preserve the intracellular milieu. This technique is infrequently used to assess the influence of metabotropic receptor agonists on the membrane potential. Somatic perforated-patch recordings revealed that muscarinic receptor activation depolarizes the membrane potential in mPFC pyramidal neurons obtained from young rats (Kurowski et al. 2015). Perforated-patch recordings from the soma also showed that a D1 receptor agonist does not influence the membrane potential in mPFC pyramidal neurons obtained from adult rats (Szulczyk et al. 2012).

It has been reported that isoproterenol increases excitatory postsynaptic transmission in the PFC via protein kinase A (Ji et al. 2008). Moreover, isoproterenol enhances calcium currents in the amygdala (Huang et al. 1998) and sodium currents in the PFC (Szulczyk 2015), also via protein kinase A. It has, however, been shown in striatal neurons that isoproterenol depolarizes the membrane potential and that only adenylate cyclase, and not protein kinase A, is involved in this effect (Pisani et al. 2003). In this study, isoproterenol-induced depolarization was abolished by an adenylate cyclase inhibitor and preserved in the presence of a protein kinase A inhibitor, which also proves that isoproterenol may induce its effects by an adenylate cyclase-dependent, but protein kinase A-independent, transduction pathway.

Somatic recordings presented herein show that isoproterenol depolarizes the membrane potential via the opening of I_h channels. It has been described that these channels are located mainly in the distal apical dendrite of layer V pyramidal neurons (Berger et al. 2001). It was thus reasonable to expect that the somatic effect may be underestimated because of the electrotonic distance between the dendrite and the soma. It has been shown in cortical pyramidal neurons in whole-cell recordings that inhibition of I_h channels results in a larger hyperpolarization in the distal than in the proximal apical dendrite because of the greater I_h channels density in the distal dendrite (Berger et al. 2001).

The results obtained in this study show that isoproterenol exerts the same effect on the membrane potential in the soma and in the apical dendrite up to 250 μm from the soma. One may thus hypothesize that in mPFC pyramidal neurons, the density of I_h channels is uniform along the first 250 μm of the dendrite.

Dendritic perforated-patch recordings are extremely rare and technically very difficult. Other authors have recorded dendritic membrane potential and action potentials in the whole-cell configuration (Berger et al. 2001, Yuan et al. 2002). In one study, however, perforated-patch recordings were made from enzymatically isolated dendritic segments. The authors proved that GABA-evoked chloride current densities are similar in the soma and in the dendrites of cortical neurons (van Brederode et al. 2001). The results presented here show that dendritic perforated-patch recordings in the slice are possible and useful in assessing the effects of metabotropic receptor activation on the electrophysiological parameters of neurons. The advantage of this electrophysiological approach is that the dendritic cytoplasm is not dialyzed, which ensures that the signal transduction pathway from the receptor to the cellular effector is not distorted.

The important feature of PFC pyramidal neurons is their persistent activity, which is important for working memory (Fuster and Alexander 1971, Thuault et al. 2013). In PFC pyramidal neurons and in other neurons, persistent activity can be generated by a brief depolarizing stimulus in the presence of a muscarinic agonist (Thuault et al. 2013, Tahvildari et al. 2007, Egorov et al. 2002). These experiments showed that tonic membrane potential hyperpolarization by a few millivolts reduces persistent firing (Thuault et al. 2013, Egorov et al. 2002). The authors also observed that tonic membrane potential depolarization by a few millivolts increases the probability of persistent activity generation (Thuault et al. 2013, Tahvildari et al. 2007). The isoproterenol-induced depolarization observed in this study may thus make it easier to generate persistent activity. Further behavioral investigations are required to confirm if this process enhances PFC cognitive functions. One may speculate, however, that the membrane potential change in itself may influence the persistent activity of PFC and consequently, affect working memory.

This study shows that a β-receptor agonist depolarizes the membrane potential in mPFC pyramidal neurons. The effect is present in the soma and in the apical dendrite. The depolarization exerted by isoproterenol depends on I_h channels. This effect is adenylate cyclase-dependent and protein kinase A-independent. In this study, data from extremely rare dendritic perforated-patch recordings were presented. Isoproterenol-induced depolarization may modulate PFC cognitive functions.

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