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Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurones of the rat

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1 Intracellular recordings were made in a pontine slice preparation of the rat brain containing the nucleus locus coeruleus (LC). The pressure application of α,β-methylene ATP (α,β-meATP) caused reproducible depolarizations which were depressed by suramin (30 μM) and abolished by suramin (100 μM). Pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 10, 30 μM) also concentration-dependently inhibited the α,β-meATP-induced depolarization, although with a much slower time-course than suramin. Almost complete inhibition developed with 30 μM PPADS. Reactive blue 2 (30 μM) did not alter the effect of α,β-meATP, while reactive blue 2 (100 μM) slightly depressed it.

2 Pressure-applied (**S**)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) also depolarized LC neurones. Kynurenic acid (500 μM) depressed and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 50 μM) abolished the response to AMPA. Suramin (100 μM) potentiated the AMPA effect.

3 Pressure-applied noradrenaline hyperpolarized LC neurones. Suramin (100 μM) did not alter the effect of noradrenaline.

4 Focal electrical stimulation evoked biphasic synaptic potentials consisting of a fast depolarization (p.s.p.) followed by a slow hyperpolarization (i.p.s.p.). A mixture of D(−)-2-amino-5-phosphonopentanoic acid (AP-5; 50 μM), CNQX (50 μM) and picrotoxin (100 μM) depressed both the p.s.p. and the i.p.s.p. Under these conditions suramin (100 μM) markedly inhibited the p.s.p., but did not alter the i.p.s.p. In the combined presence of AP-5 (50 μM), CNQX (50 μM), picrotoxin (100 μM), strychnine (0.1 μM), tropisetron (0.5 μM) and hexamethonium (100 μM), a high concentration of suramin (300 μM) almost abolished the p.s.p. without changing the i.p.s.p.

5 In the presence of kynurenic acid (500 μM) and picrotoxin (100 μM), PPADS (30 μM) depressed the p.s.p. Moreover, the application of suramin (100 μM) to the PPADS (30 μM)-containing medium failed to cause any further inhibition. Neither PPADS (30 μM) nor suramin (100 μM) altered the i.p.s.p.

6 It was concluded that the cell somata of LC neurones are endowed with excitatory P2-purinoceptors. ATP may be released either as the sole transmitter from purinergic neurones terminating at the LC or as a co-transmitter of noradrenaline from recurrent axon collaterals or dendrites of the LC neurones themselves.

Keywords: ATP; noradrenaline; locus coeruleus

Introduction

Adenosine 5′-triphosphate (ATP) has been suggested to be a transmitter in purinergic neurones of the peripheral nervous system as well as a co-transmitter with noradrenaline in postganglionic sympathetic neurones (Burnstock, 1986; von Kügelgen & Starke, 1991; Zimmermann, 1994). Thereby, ATP produces excitatory junction potentials and subsequent contractions of smooth muscle cells. However, ATP is also a fast neuro-neuronal transmitter in fibre networks formed by cultured sympathetic neurones of guinea-pig coeliac ganglia kept in tissue culture (Evans *et al*., 1992; Silinsky & Gerzanich, 1993). Moreover, ATP mediates, in addition to acetylcholine, synaptic transmission in myenteric neurones of the guinea-pig ileum (Galligan & Bertrand, 1994; Zhou & Galligan, 1996). ATP appears to act at two types of P2-purinoceptor belonging either to the P2X (ligand-activated cationic channel) or P2Y (G protein-coupled receptor) types (Abbracchio & Burnstock, 1994; Fredholm *et al*., 1994). Fast responses of smooth muscle and neurones are mediated by P2X purinoceptors (Bean, 1992; Illes & Nörenberg, 1993).

Although both mRNA measurements (Kidd *et al*., 1995; Surprenant *et al*., 1995) and electrophysiological studies (Illes & Nörenberg, 1993; Surprenant *et al*., 1995) indicate that P2X purinoceptors are widely distributed in the central nervous system, functional evidence that ATP acts as an excitatory transmitter has only been obtained for neurones of the medial habenula (Edwards *et al*., 1992). P2X purinoceptor-channels were found at neurones of the medial habenula (Edwards *et al*., 1992), locus coeruleus (Harms *et al*., 1992; Shen & North, 1993), tuberomamillary nucleus (Furukawa *et al*., 1994), supraoptic nucleus (Hiruma & Bourque, 1995) and dorsal motor nucleus of the vagus (Nabekura *et al*., 1995).

Focal electrical stimulation evokes in central noradrenergic neurones of the locus coeruleus (LC) biphasic synaptic potentials, consisting of early depolarizing (p.s.p) and late hyperpolarizing (i.p.s.p.) components. It has been found that the p.s.p. is due to the release of glutamate from afferent fibres predominantly onto non-*N*-methyl-D-aspartate (NMDA) receptors and of γ-aminobutyric acid (GABA) onto $GABA_A$ receptors (Cherubini *et al*., 1988; Williams *et al*., 1991). A smaller part of the p.s.p. is glycine-mediated (Williams *et al*., 1991). The i.p.s.p. is due to the release of noradrenaline onto α_2-adrenoceptors either from recurrent axon collaterals or dendrites of the LC neurones themselves or from afferent fibres originating in the nucleus paragigantocellularis (Egan *et al*., 1983; Williams *et al*., 1991). The aim of the present experiments was to find out whether there is a purinergic component of the p.s.p., indicating a fast transmitter function of ATP in the LC. For this purpose the interaction between P2-purinoceptor antagonists (suramin, reactive blue 2, pyridoxal-phosphate-6-azophenyl-2,4-disulphonic acid (PPADS) was investigated with the P2-purinoceptor agonist α,β-methylene ATP (α,β-meATP) and the non-NMDA agonist (**S**)-α-amino-

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3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Thereafter, it has been demonstrated that suramin and PPADS inhibit the p.s.p. in concentrations which abolish the depolarization of LC neurones induced by α,β-meATP but not by AMPA.

Methods

Brain slice preparation

Mid-pontine slices of the rat brain were prepared and maintained as previously described (Nieber *et al*., 1995). In brief, male Wistar rats (150–200 g) were anaesthetized with ether and decapitated. Coronal slices about 400 μm thick, containing the caudal part of the LC, were prepared in oxygenated medium at 1–4°C with a Lancer vibratome. A single slice was placed in a recording chamber and superfused at a rate of 2 ml min^{-1} with medium saturated with 95% O_2-5% CO_2 and kept at 35–36°C. The medium was composed of (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 25 and glucose 11. Ascorbic acid (0.3 mM) and EDTA (0.03 mM) were present in order to prevent the oxidation of noradrenaline.

Identification of LC neurones and intracellular recording

Locus coeruleus neurones were identified under a binocular microscope at the ventrolateral border of the fourth ventricle. They were distinguished from neighbouring mesencephalic trigeminal neurones by their electrophysiological properties, including spontaneous firing at a frequency of 0.2–5 Hz and a hyperpolarizing response to noradrenaline (Nieber *et al*., 1995).

Recording of the membrane potential and current injection was carried out with glass microelectrodes filled with KCl (2 M; tip resistance, 60–100 MΩ) using a high impedance preamplifier and a bridge circuit (Axoclamp-2A; Axon Instruments). In order to prevent spontaneous firing, the membrane potential of LC neurones was raised by about 20 mV from rest by injecting a constant hyperpolarizing current. The membrane potential was displayed on a Gould RS 3200 pen recorder and was determined on withdrawal of the microelectrode from the cell at the end of each experiment.

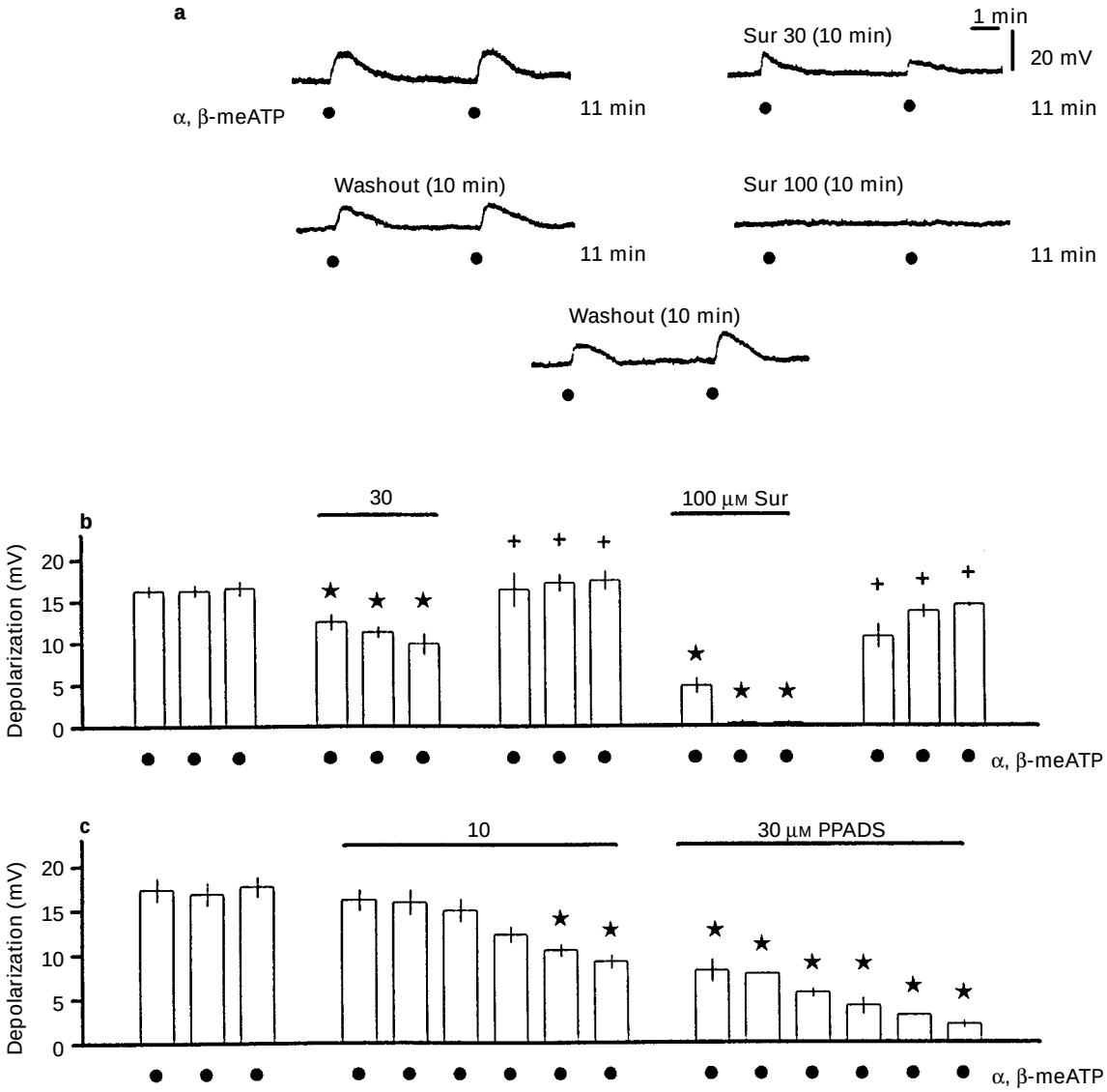

Figure 1 Depolarization of rat LC neurones by pressure application of α,β-meATP and interaction with suramin or PPADS. (a, b) Inhibition by suramin of responses to α,β-meATP. (a) Representative tracings. α,β-meATP was applied at regular intervals (•). All concentrations are expressed in μM. The superfusion and washout times of suramin (Sur) are in parentheses. The periods between traces are indicated. (b) Means±s.e.mean of 5 experiments similar to those shown in (a). (c) Inhibition by PPADS of responses to α,β-meATP. Means±s.e.mean of 6 experiments. Antagonists were present in the superfusion medium over the periods marked by the horizontal bars. $^*P<0.05$; significant differences from the effect of α,β-meATP before antagonist application. $+P<0.05$; significant differences from the effect of α,β-meATP immediately before the washout of suramin.

Application of drugs and electrical stimulation

All agonists (α,β-meATP 10 mM, AMPA 1 mM, noradrenaline 100 μM) were applied by pressure pulses (33 kPa) from a micropipette (tip diameter, 10–20 μm). The duration of the pulses (α,β-meATP, noradrenaline, 40–640 ms; AMPA, 4–70 ms) was adjusted so that the amplitude of the change in membrane potential was about 15 mV. Agonists were applied every 5 min throughout, except when superfusion with an antagonist-containing medium was started or ceased. All antagonists were applied by changing the superfusion medium by means of three-way taps. At a constant flow rate of 2 ml min^{-1} about 30 s were required to reach the bath.

Synaptic potentials were evoked by electrical stimulation (0.1 Hz, 0.1–0.4 ms, 30–140 V) with bipolar tungsten electrodes inserted into the slice at a distance of about 100 μm from the site of recording. Under these conditions, the amplitudes of the p.s.ps and i.p.s.ps were about 15 and 3 mV, respectively. Four synaptic potentials were averaged. In these experiments, the membrane potential was fed to a digital tape-recorder (Biologic DTR-1200) for further print-out on an X-Y plotter (Gould Colorwriter 6120) and was analysed from that.

The effects of P2 purinoceptor antagonists on membrane potential shifts induced by pressure-applied agonists were evaluated as percentage changes. Each antagonist concentration was added to the superfusion medium 5 min before agonist application for 15 min (suramin, reactive blue 2) or 35 min (PPADS) in total. The depolarizing or hyperpolarizing responses to pressure applied agonists were measured before antagonist application and were than compared with responses to agonists measured after 15 min (suramin) or 35 min (PPADS) superfusion with antagonists. In additional experiments kynurenic acid or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was applied and their effects were calculated after 15 min superfusion with respect to the pre-antagonist amplitude of agonist-induced depolarizations. In some experiments, noradrenaline was used to hyperpolarize LC neurones both before and 15 min after starting the superfusion of suramin.

The effects of P2-purinoceptor antagonists on synaptic potentials were also evaluated as percentage changes. At first the

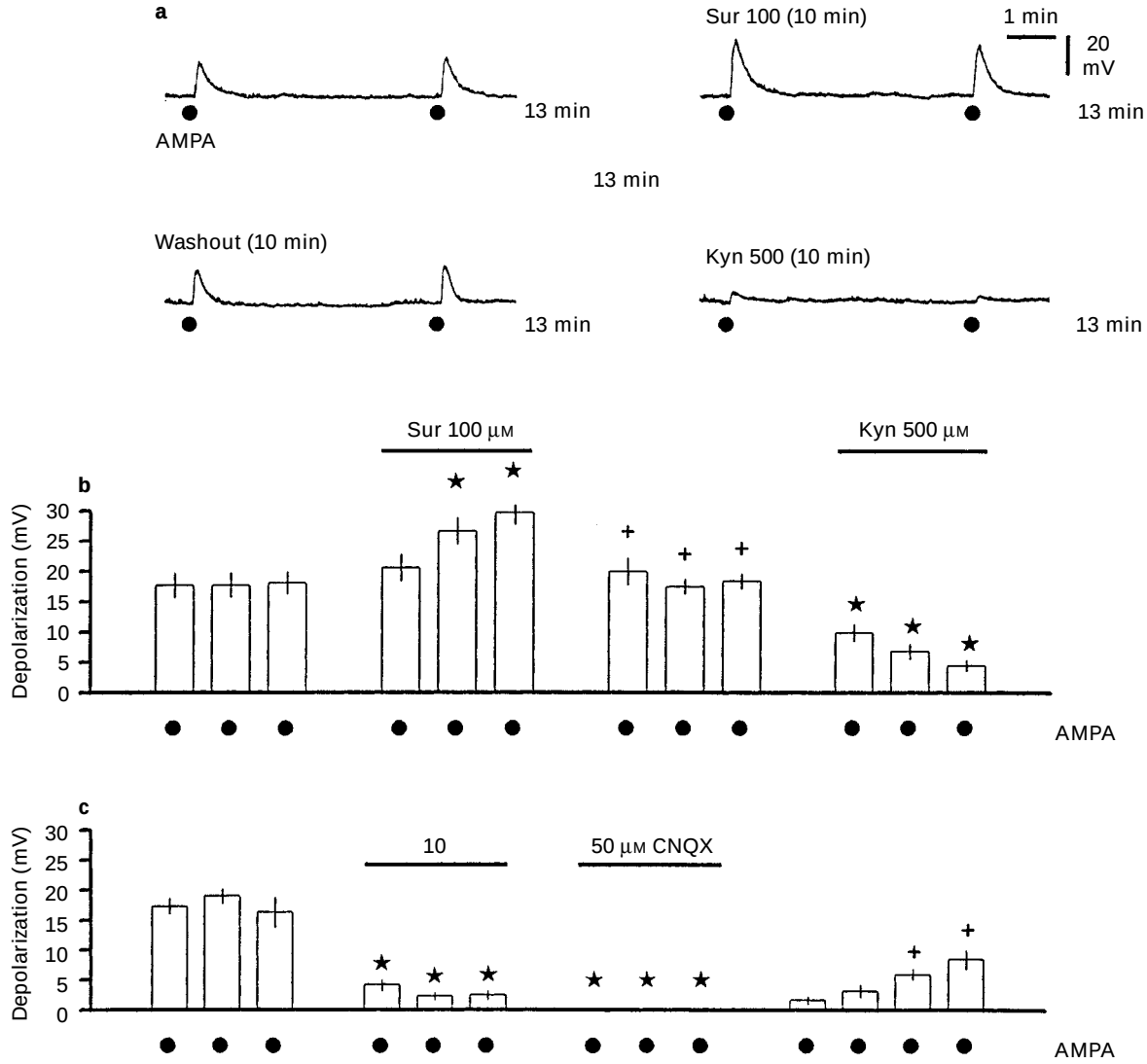

Figure 2 Depolarization of rat LC neurones by pressure application of AMPA and interaction with suramin, kynurenic acid or CNQX. (a, b) Potentiation by suramin and inhibition by kynurenic acid of responses to AMPA. (a) Representative tracings. AMPA was applied at regular intervals (●). All concentrations are expressed in μM. The superfusion and washout times of suramin (Sur) and kynurenic acid (Kyn) are in parentheses. The periods between traces are indicated. (b) Means ± s.e.mean of 5 experiments similar to those shown in (a). (c) Inhibition by CNQX of responses to AMPA. Means ± s.e.mean of 5 experiments. Antagonists were present in the superfusion medium over the periods marked by the horizontal bars. $^*P<0.05$; significant differences from the effect of AMPA before antagonist application. $+P<0.05$; significant differences from the effect of AMPA immediately before the washout of suramin or CNQX.

excitatory amino acid receptor- (kynurenic acid or D(−)-2-amino-5-phosphonopentanoic acid (AP-5) and CNQX) and $GABA_A$ receptor- (picrotoxin) mediated components of the p.s.ps were blocked by the respective antagonists. Subsequently, the amplitude of 4 averaged synaptic potentials was measured in the absence of P2-purinoceptor antagonists and was then compared with the amplitude of 4 averaged synaptic potentials measured after 10 min (suramin) or 30 min (PPADS) superfusion with antagonists. In separate experiments, the effect of suramin was determined before and 20 min after the application of an antagonist cocktail (see Figure 5a) and expressed as percentage inhibition.

Materials

The following drugs were used: suramin hexasodium salt (Bayer, Wuppertal, Germany; tropisetron (A. Surprenant, Glaxo, Geneva, Switzerland); (−)-noradrenaline hydrochloride (Hoechst, Frankfurt am Main, Germany); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(−)-2-amino-5-phosphonopentanoic acid (AP-5), reactive blue 2, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium salt (PPADS; Dr Lambrecht, Biozentrum der Universität, Frankfurt, Germany); α,β-methyleneadenosine 5′-triphosphate dilithium salt, hexamethonium chloride, kynurenic acid, picrotoxin, strychnine hydrochloride (Sigma, Deisenhofen, Germany); (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; Tocris Cookson, Bristol, U.K.).

Stock solutions (1–10 mM) of all drugs except AMPA (0.5 M HCl) and CNQX (dimethyl sulphoxide) were prepared in distilled water. Further dilutions were made in medium. The highest concentrations of suramin (100, 300 μM) were directly dissolved in medium. Equivalent quantities of the solvent had no effect.

Statistics

Means ± s.e.mean are given throughout. Multiple comparisons with a control value were performed by one-way analysis of variance followed either by non-parametric Dunn's test or by parametric Bonferroni's *t* test, as appropriate. All other comparisons with a control value were made by paired Student's *t* test. Percentage changes were compared with zero by paired Student's *t* test. A probability level of 0.05 or less was considered to be statistically significant.

Results

A total of fifty-nine LC neurones included in this study had a resting membrane potential of 52.3 ± 1.3 mV. The generation of spontaneous action potentials was prevented by passing continuous hyperpolarizing current via the recording electrode.

Interaction of P2-purinoceptor and excitatory amino acid (EAA) receptor agonists with their antagonists

When the enzymatically stable P2-purinoceptor agonist α,β-meATP was applied by pressure from a micro-pipette located near the recording electrode, LC neurones responded with reproducible depolarizations (Figure 1). The reversible P2-purinoceptor antagonist suramin depressed responses to α,β-meATP at 30 μM and abolished them at 100 μM (Figure 1a and b). The percentage inhibition was 40.4 ± 7.4% and 98.4 ± 1.6% ($n=5$; $P<0.05$). The effects of both suramin concentrations were rapidly reversed on washout. The pseudo-irreversible P2-purinoceptor antagonist PPADS caused a slowly developing and concentration-dependent inhibition of the α,β-meATP-induced depolarization (Figure 1c). This inhibition was 47.8 ± 3.3% ($P<0.05$) and 87.4 ± 1.8% ($P<0.05$; $n=6$ each), at 10 and 30 μM PPADS, respectively. A lower concentration of another P2-purinoceptor antagonist, reactive blue 2 (30 μM) did not alter the effect of α,β-meATP (1.4 ± 5.4%; $P>0.05$), while a higher concentration (100 μM) of this compound slightly depressed it (29.6 ± 4.0%; $P<0.05$; $n=6$ each).

The pressure-ejected non-NMDA agonist AMPA depolarized LC neurones with a faster time-course than α,β-meATP (Figure 2). When AMPA was applied according to a protocol identical to that used previously for α,β-meATP, suramin (100 μM) potentiated the effect of AMPA by 71.7 ± 10.3% ($n=5$; $P<0.05$) (Figure 2a and b). This potentiation was fully reversible on washout. The mixed NMDA and non-NMDA antagonist kynurenic acid (500 μM) (64.5 ± 10.2%; $n=5$; $P<0.05$) (Figure 2b) and a lower concentration (10 μM) of the non-NMDA antagonist CNQX (89.1 ± 4.1%; $n=5$; $P<0.05$) (Figure 2c) depressed the responses to AMPA. By contrast, CNQX at a higher concentration (50 μM) abolished the AMPA-induced depolarization (Figure 2c).

Pressure application of the mixed α_1- and α_2-adrenoceptor agonist noradrenaline hyperpolarized LC neurones with a rather slow time-course (Figure 3). Suramin (100 μM) did not alter the effect of noradrenaline (−2.0 ± 2.0%; $n=5$; $P>0.05$) (Figure 3).

Effects of P2-purinoceptor antagonists on synaptic potentials in the presence of EAA and $GABA_A$ receptor antagonists

Biphasic synaptic potentials were evoked by electrical stimulation (Figure 4a). NMDA, non-NMDA and $GABA_A$ receptors were blocked by AP-5 (50 μM), CNQX (50 μM) and picrotoxin (100 μM), respectively. Under these conditions both the p.s.p. (72.8 ± 4.2%; $P<0.05$) and i.p.s.p. (60.0 ± 5.0%; $n=5$ each; $P<0.05$) amplitudes were reduced (Figure 4). A further application of suramin (100 μM) reversibly depressed the p.s.p. by 63.8 ± 12.0% ($P<0.05$), but did not alter the i.p.s.p. When kynurenic acid (500 μM) was used instead of AP-

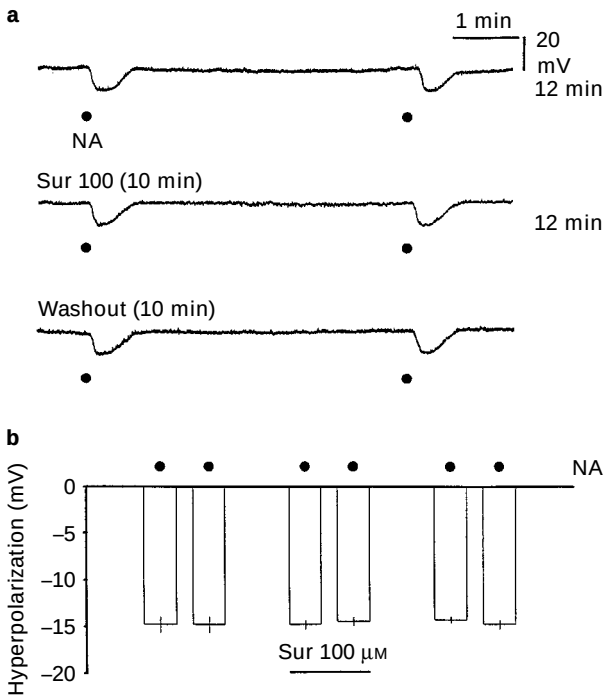

Figure 3 Hyperpolarization of rat LC neurones by pressure application of noradrenaline and no interaction with suramin. (a) Representative tracings. Noradrenaline (NA) was applied at regular intervals (●). All concentrations are expressed in μM. The superfusion and washout times of suramin (Sur) are in parentheses. The periods between traces are indicated. Means ± s.e.mean of 5 experiments similar to those shown in (a). Antagonists were present in the superfusion medium over the period marked by the horizontal bar.

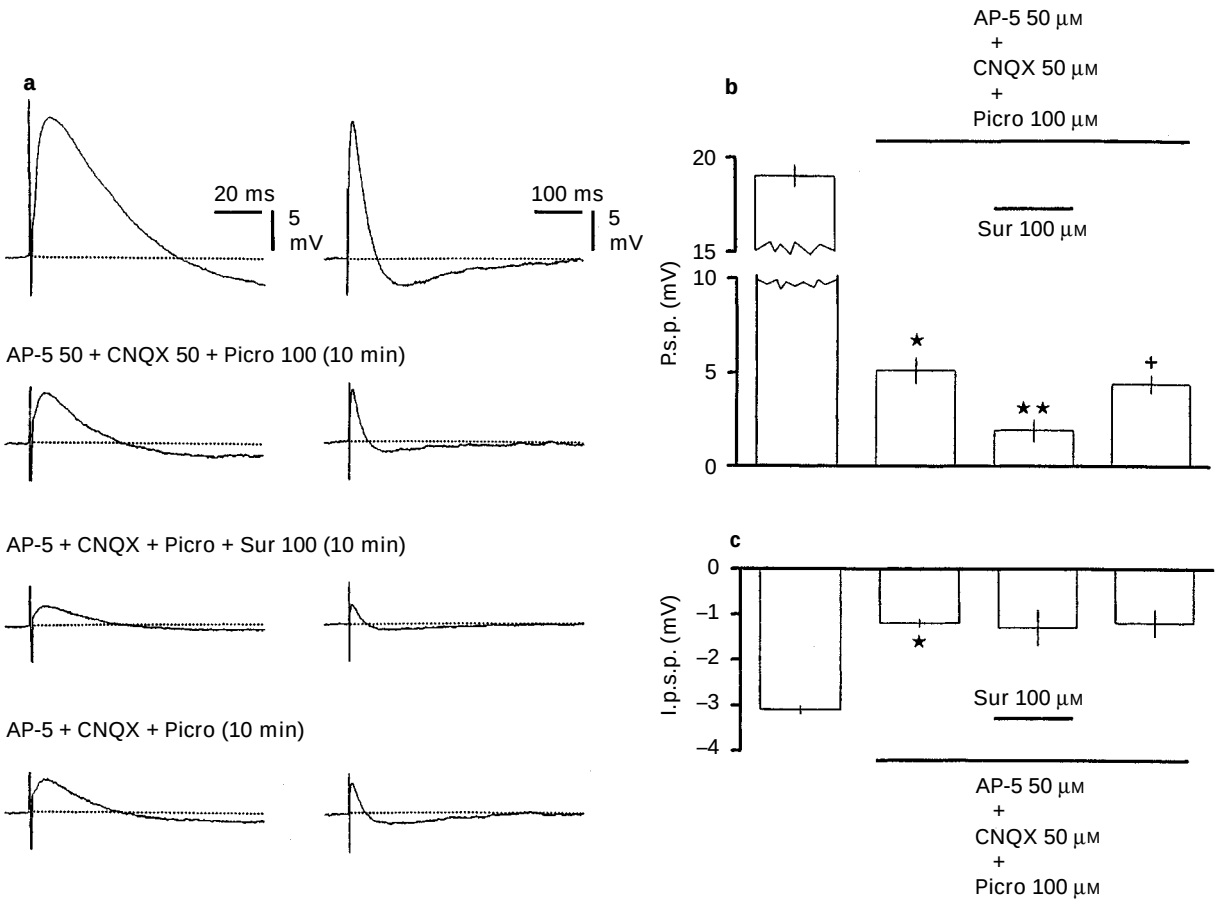

Figure 4 Effects of AP-5, CNQX, picrotoxin (Picro) and suramin (Sur) on p.s.p./i.p.s.p. sequences evoked by focal electrical stimulation in rat LC neurones. (a) Representative tracings recorded with two different time scales. The dotted lines indicate the membrane potential. All concentrations are expressed in μM. The superfusion times of antagonists are in parentheses. (b, c) Means $\pm$ s.e.mean of 5 experiments similar to those shown in (a). (b) Effects on p.s.p. amplitudes. (c) Effects on i.p.s.p. amplitudes. Antagonists were present in the superfusion medium over the periods marked by the horizontal bars. $*P<0.05$; significant differences from synaptic potentials measured before the application of AP-5, CNQX and picrotoxin. $**P<0.05$; significant difference from synaptic potentials measured before the application of suramin. $+P<0.05$; significant difference from synaptic potentials measured before the washout of suramin.

5 and CNQX, the p.s.p. ($51.7\pm8.1\%$; $P<0.05$), but not the i.p.s.p. ($13.5\pm12.8\%$; $n=7$; $P>0.05$) amplitudes decreased. Suramin at 30 and 100 μM produced a concentration-dependent inhibition of the residual p.s.p. ($22.2\pm3.6\%$ and $45.1\pm7.8\%$, respectively; $n=7$; $P<0.05$). By contrast, only the higher concentration (100 μM) of suramin caused some depression of the i.p.s.p. ($29.1\pm7.6\%$; $n=7$; $P<0.05$). The effects of suramin (30, 100 μM) on the p.s.p. were completely reversible on washout.

A cocktail of antagonists was applied in order to isolate the P2-purinoceptor-mediated part of the p.s.p. by the blockade of various ligand-activated cationic channels (NMDA, non-NMDA, $GABA_A$, glycine, 5-HT_3 and nicotinic acetylcholine receptors; Figure 5a). A mixture of AP-5 (50 μM), CNQX (50 μM), picrotoxin (100 μM), strychnine (0.1 μM), tropisetron (0.5 μM) and hexamethonium (100 μM) was used. The residual fraction of the p.s.p. was strongly inhibited by suramin (300 μM) ($80.5\pm4.3\%$; $P<0.05$), while the i.p.s.p. was not altered ($33.9\pm17.6\%$; $n=5$ each; $P>0.05$). In the presence of kynurenic acid (500 μM) and picrotoxin (100 μM), PPADS (30 μM) depressed the p.s.p. ($45.9\pm8.4\%$; $n=5$; $P<0.05$) (Figure 5b and c) as much as suramin (100 μM) did in previous experiments. Moreover, the application of suramin (100 μM) to the PPADS (30 μM) - containing superfusion medium failed to cause any further inhibition of the p.s.p. (Figure 5c). The effect of PPADS (30 μM) did not reverse after washout. Finally, neither PPADS (30 μM) nor suramin (100 μM) altered the i.p.s.p. (Figure 5d).

Discussion

Equipotent concentrations of the enzymatically stable analogue α,β-meATP depolarized LC neurones with a slower time-course than the EAA receptor agonist AMPA. Therefore, in agreement with previous work, α,β-meATP appears to activate both P2X- and P2Y-purinoceptors of these neurones by producing a fast and slow response, respectively (Harms *et al.*, 1992; Shen & North, 1993; Illes *et al.*, 1995). AMPA stimulates an ionotropic glutamate receptor-type and causes a fast response only. The mode of action of P2-purinoceptors is the opening of non-selective cationic channels (P2X) and the closure of potassium channels (P2Y). Since LC neurones were routinely hyperpolarized to about -80 mV, the cationic current will predominate under these experimental conditions. However, the onset of responses to α,β-meATP is known to vary with its concentration (e.g. Khakh *et al.*, 1997) and on the basis of these experiments the possibility that the effects of α,β-meATP and AMPA are not being compared at the same points of their respective concentration-response curves cannot be excluded.

It was suggested previously (Fröhlich *et al.*, 1996) that rat LC neurones, like rat superior cervical ganglion neurones, possess purinoceptors identical to the $P2X_2$ receptor recently isolated by molecular biological methods from rat PC12 cells (Brake *et al.*, 1994; Surprenant *et al.*, 1995). This receptor has a rather low sensitivity to ATP and especially to α,β-meATP, exhibits little desensitization to P2-purinoceptor agonists

(Brake *et al*., 1994; Surprenant *et al*., 1995) and its mRNA is heavily expressed in the LC (Collo *et al*., 1996). In view of the effect of α,β-meATP on LC neurones, a heteropolymerization of the $P2X_2$ subunit with another subunit is likely. $P2X_1$ receptors isolated from the rat vas deferens show a higher sensitivity to α,β-meATP and are easily desensitized (Valera *et al*., 1994). $P2X_4$ receptors isolated from the rat superior cervical ganglion are not blocked by suramin (Buell *et al*., 1996). Finally, LC neurones may possess a G protein-coupled P2Y-purinoceptor similar to the recently isolated P2Y1 receptor (Webb *et al*., 1993; Barnard *et al*., 1994).

It was not possible to differentiate the P2X- and P2Y-purinoceptor-mediated current components with the P2-purinoceptor antagonists. Suramin and reactive blue 2 do not discriminate between P2-purinoceptor-types (Kennedy, 1990; Kennedy & Leff, 1995; Humphrey *et al*., 1995). Accordingly, suramin strongly and reversibly depressed the effect of α,β-meATP in a range of concentrations (30–100 μM) that also

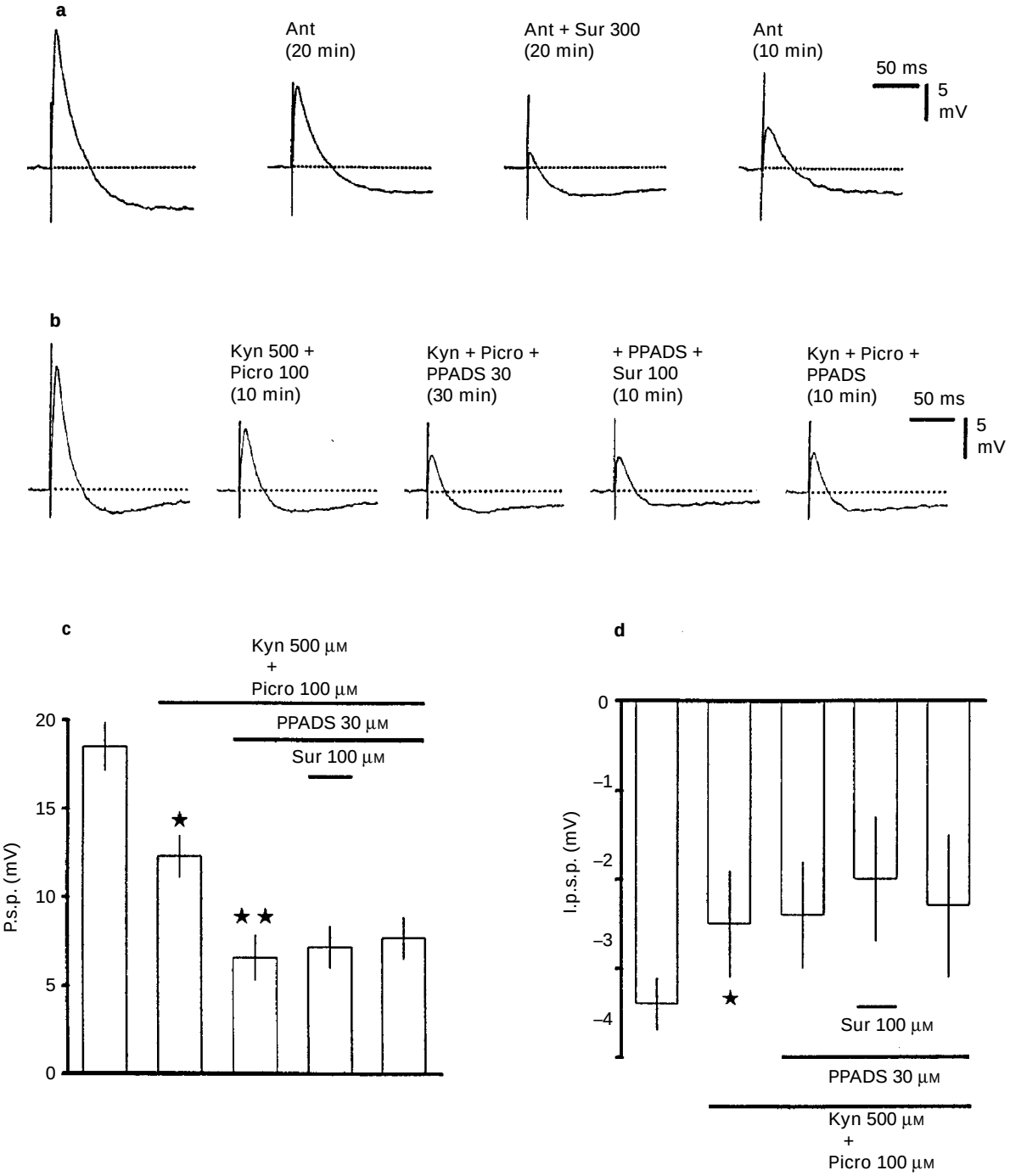

Figure 5 Effects of a cocktail of antagonists or PPADS and suramin on p.s.p./i.p.s.p. sequences evoked by focal electrical stimulation in rat LC neurones. The dotted lines indicate the membrane potential. All concentrations are expressed in μM. The superfusion times of antagonists are in parentheses. (a) Pharmacological isolation of an almost totally suramin (Sur)-sensitive purinergic p.s.p. Representative tracings out of 5 similar experiments. AP-5 (50 μM), CNQX (50 μM), picrotoxin (100 μM), strychnine (0.1 μM), tropisetron (0.5 μM) and hexamethonium (100 μM) were used as antagonists (Ant). (b, c, d) Inhibition by PPADS, alone or in combination with suramin, of the p.s.p. amplitudes; kynurenic acid (Kyn) and picrotoxin (Picro) were present in the bath. (b) Representative tracings. (c) Effects on p.s.p. amplitudes. (d) Effects on i.p.s.p. amplitudes. Means $\pm$ s.e.mean of 5 experiments similar to those shown in (a). Antagonists were present in the superfusion medium over the periods marked by the horizontal bars. $^*P<0.05$; significant differences from synaptic potentials evoked before the application of kynurenic acid and picrotoxin. $^{**}P<0.05$; significant difference from synaptic potentials evoked before the application of PPADS.

inhibit the contractile effect of this nucleotide in smooth muscle organs (Bültmann & Starke, 1994). By contrast, reactive blue 2 only slightly affected with the α,β-meATP responses (see also Fröhlich *et al*., 1996). PPADS in a concentration (30 μM) which is supposed to be selective for P2X-purinoceptors (Ziganshin *et al*., 1994 Humphrey *et al*., 1995) caused a slowly developing and almost complete inhibition of the α,β-meATP responses. The slow time-course and pseudo-irreversibility of the blockade may be due to the formation of Schiff bases with lysine residues in the P2X-purinoceptor (Humphrey *et al*., 1995; Buell *et al*., 1996).

Suramin, but not PPADS is known to depress the glutamate-induced current in CA1 pyramidal cells of rat hippocampal slices (Motin & Bennett, 1995). Moreover, suramin inhibited currents evoked by kainate, NMDA and GABA in rat cultured hippocampal neurones (Nakazawa *et al*., 1995) and reduced the dimethylphenylpiperazinium-induced release of noradrenaline from chick sympathetic neurones also kept in culture (Allgaier *et al*., 1995; Humphrey *et al*., 1996). In the present study, suramin potentiated rather than inhibited the depolarizing effect of AMPA. The mechanism of this potentiation is unknown but exemplifies another effect of suramin not confined to P2-purinoceptors. Finally, suramin did not interfere with the hyperpolarizing effect of noradrenaline.

Focal electrical stimulation evoked p.s.ps with a short synaptic delay and a fast rise-time (see e.g. Figure 4a). A blockade of ionotropic EAA receptors by kynurenic acid (or by AP-5 and CNQX to block NMDA and non-NMDA receptors, respectively) and $GABA_A$ receptors by picrotoxin considerably reduced the p.s.p. (see also Cherubini *et al*., 1988 Williams *et al*., 1991). Hence, a large fraction of the p.s.p. is due to the release of glutamate and GABA from afferent fibres. Although GABA is an inhibitory transmitter in the LC, it produces depolarization when KCl electrodes are used for recording. Under these conditions the reversal potential of Cl^- is lower than the resting membrane potential (Cherubini *et al*., 1988; Osmanovic & Shefner, 1990).

It is noteworthy that kynurenic acid (500 μM) was applied in a concentration which is known to block the depolarizing effect of glutamate in LC neurones (Cherubini *et al*., 1988). Since responses to AMPA were abolished by CNQX (50 μM), but not by kynurenic acid (500 μM), a combination of CNQX (50 μM) and AP-5 (50 μM) was used in some experiments to exclude completely the EAA receptor-mediated part of the p.s.p.

A considerable fraction of the i.p.s.p. is due to the release of noradrenaline either from recurrent axon collaterals or dendrites of the LC neurones themselves or from fibres afferent to the LC (Egan *et al*., 1983; Williams *et al*., 1991). AP-5 (50 μM) in combination with a high concentration of CNQX (50 μM) inhibited both the p.s.p. and the i.p.s.p. Kynurenic acid (500 μM) also depressed the p.s.p., but its inhibitory effect on the i.p.s.p. was rather small and not consistent. It is possible that the release of noradrenaline is facilitated by endogenous glutamate acting via NMDA and non-NMDA receptors (Fink & Göthert, 1990). Consequently, the i.p.s.p. may be depressed by antagonists for these receptors by excluding an ongoing facilitation. Although picrotoxin (100 μM) was also co-applied with the EAA receptor antagonists, it is highly unlikely that this compound interferes with the i.p.s.p.

In the presence of antagonists for NMDA, non-NMDA and $GABA_A$ receptors, the p.s.p. was reversibly inhibited by a concentration of suramin (100 μM) that abolished the depolarizing effect of α,β-meATP. Although suramin depressed responses to α,β-meATP, it potentiated responses to AMPA. Since NMDA receptor antagonists have little effect on the p.s.p. amplitude, while non-NMDA receptor antagonists clearly reduce it, the effect of glutamate released on nerve stimulation may be mediated by this latter receptor-type (Cherubini *et al*., 1988). Hence, two pieces of evidence strongly suggest that the effect of suramin on the p.s.p. is due to antagonism at P2-purinoceptors rather than to a non-selective interaction with EAA receptors. Firstly, an AMPA receptor-mediated fraction of the p.s.p. is unlikely to persist in the presence of CNQX (50 μM) which abolished the effect of exogenous AMPA. Secondly, even if such a fraction existed, suramin would potentiate rather than inhibit it.

In agreement with the assumption that there is a purinergic fraction of the p.s.p., PPADS a P2-purinoceptor antagonist that did not alter the facilitatory effect of AMPA on the firing rate of LC neurones (Fröhlich *et al*., 1996), also depressed the p.s.p. A clear indication for a common site of action of suramin and PPADS at the recognition site of the P2-purinoceptor is the non-additivity of the inhibitory effects of these compounds.

Even after exclusion of the fast responses to glutamate, GABA and ATP with their respective antagonists, a small fraction of the p.s.p. persisted. However, it disappeared almost completely when the excitatory effects of additional fast transmitters (glycine, 5-hydroxytryptamine, acetylcholine) were inhibited by a mixture of antagonists and, in addition, the concentration of suramin was increased from 100 to 300 μM (see also Bültmann & Starke, 1994).

An adenosine deaminase-containing pathway has been identified, projecting from the posterior hypothalamus to the mesencephalic trigeminal nucleus (MNV) in the immediate neighbourhood of the LC (Nagy *et al*., 1986). It is conceivable that this pathway releases ATP as a neurotransmitter onto its target cells. While MNV neurones responded to ATP with an inward current (Khakh *et al*., 1997), they were insensitive to the degradation product adenosine (Regenold *et al*., 1988). LC neurones responded to ATP and adenosine with inward (Harms *et al*., 1992; Shen & North, 1993) and outward currents (Regenold & Illes, 1990; Nieber *et al*., 1995), respectively. Hence, ATP may be involved in neurotransmission from a hitherto unidentified afferent purinergic pathway to the LC.

Alternatively, ATP and noradrenaline may be co-released from dendrites or recurrent axon collaterals onto LC neurones producing excitation (p.s.p.) and inhibition (i.p.s.p.), respectively. Since the ratio of ATP to noradrenaline varies with different stimulation conditions (von Kügelgen & Starke, 1991), a finely tuned regulation of the neuronal firing rate may result. This situation is different from that observed in postganglionic sympathetic nerves, where both ATP and noradrenaline are excitatory transmitters (Stjärne, 1989; von Kügelgen & Starke, 1991). Such additive effects of co-transmitters are the usual pattern, although subtractive effects also occur (Kupfermann, 1991). The present experiments do not allow us to determine whether ATP is released from purinergic neurones terminating at the LC or from recurrent axon collaterals or dendrites of the LC neurones themselves.

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