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Direct inhibition of the *N*-methyl-D-aspartate receptor channel by dopamine and (+)-SKF38393

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1 Dopamine is known to modulate glutamatergic synaptic transmission in the retina and in several brain regions by activating specific G-protein-coupled receptors. We have examined the possibility of a different type of mechanism for this modulation, one involving direct interaction of dopamine with ionotropic glutamate receptors.

2 Ionic currents induced by fast application of *N*-methyl-D-aspartate (NMDA) were recorded under whole-cell patch-clamp in cultured striatal, thalamic and hippocampal neurons of the rat and in retinal neurons of the chick. Dopamine at concentrations above 100 μM inhibited the NMDA response in all four neuron types, exhibiting an IC_{50} of 1.2 mM in hippocampal neurons. The time course of this inhibition was fast, developing in less than 100 ms.

3 The D_1 receptor agonist (+)-SKF38393 mimicked the effect of dopamine, with an IC_{50} of 58.9 μM on the NMDA response, while the enantiomer (−)-SKF38393 was ineffective at 50 μM. However, the D_1 antagonist R(+)-SCH23390 did not prevent the inhibitory effect of (+)-SKF38393.

4 The degree of inhibition by dopamine and (+)-SKF38393 depended on transmembrane voltage, increasing 2.7 times with a hyperpolarization of about 80 mV. The voltage-dependent block by dopamine was also observed in the presence of $MgCl_2$ 1 mM.

5 Single-channel recordings showed that the open times of NMDA-gated channels were shortened by (+)-SKF38393.

6 These data suggested that the site to which the drugs bound to produce the inhibitory effect was distinct from the classical D_1-type dopamine receptor sites, possibly being located inside the NMDA channel pore. It is concluded that dopamine and (+)-SKF38393 are NMDA channel ligands.

Keywords: Dopamine; glutamate; NMDA receptor; SKF38393; ion channel

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; cyclic AMP, adenosine 3′:5′-cyclic monophosphate; GABA, γ-aminobutyric acid; HEPES, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid]; NMDA, *N*-methyl-D-aspartate

Introduction

Dopamine is a chemical messenger that can behave as either a neurotransmitter or a neuromodulator in different regions of the central nervous system. Part of the effects of endogenous dopamine involves modulation of glutamate receptor function; this seems to be the case in the neostriatum, where dopamine can be released in close proximity to glutamatergic synapses (Freund *et al*., 1984; Kornhuber & Kornhuber, 1983), and possibly in other brain regions and in the retina. Both facilitation and inhibition of ionotropic glutamate responses by dopamine have been described, even in the same preparation (e.g., Akaike *et al*., 1987). These opposing effects can be interpreted in terms of specific modulation of the different subtypes of glutamate receptor-channels, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) or kainate, through either D_1 or D_2-type dopamine receptors. The two dopamine receptor subtypes include five different gene products that belong to the G-protein-coupled receptor superfamily and can be distinguished by selective agonists and antagonists or by functional assays (reviewed in Sokoloff & Schwartz, 1995). D_1-type receptors stimulate adenylate cyclase, increasing adenosine 3′:5′-cyclic monophosphate (cyclic AMP) production, and include the D_1 and D_5 gene products. Activation by dopamine of D_1-type receptors increases the activity of kainate channels in chick spinal motoneurons (Smith *et al*., 1995), potentiates non-NMDA glutamatergic transmission between photoreceptor and horizontal cells in fish retina (Knapp & Dowling, 1987; Knapp *et al*., 1990; Schmidt *et al*., 1994) and potentiates NMDA responses in striatal neurons (Cepeda *et al*., 1993), but depresses excitatory post-synaptic currents in rat nucleus accumbens neurons (Harvey & Lacey, 1996). These D_1 effects can usually be reproduced by application of 8-Br-cyclic AMP or forskolin, confirming the involvement of the cyclic AMP cascade (but see Harvey & Lacey, 1996). The D_2-type receptors include the D_2 gene product, which in many preparations mediates inhibition of adenylate cyclase, and the D_3 and D_4 products, whose functional mechanism is still under investigation. Thus, in most cases studied in detail, the mechanism of modulation of glutamatergic transmission by dopamine is metabotropic, mediated by G-protein-dependent intracellular effectors.

However, we recently described an effect of dopamine that does not seem to involve any of the known receptors or metabotropic pathways. The release of γ-aminobutyric acid (GABA) by chick retinal neurons in response to NMDA is inhibited by dopamine and by (+)-SKF38393, but this effect is neither mimicked by another D_1 agonist, SKF81297, nor

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blocked by the D_1 antagonist SCH23390 (Castro *et al*., 1995; do Nascimento *et al*., 1998). These and other data led us to suggest that dopamine and (+)-SKF38393 were inhibiting the NMDA response through a novel site, perhaps located on the NMDA receptor-channel complex itself. To test this hypothesis, the effect of the dopaminergic agents on the NMDA channel was studied directly by whole-cell and single-channel current recordings. Based on data obtained from retinal, striatal, thalamic and hippocampal neurons, it is shown here that dopamine and (+)-SKF38393 indeed bind to the NMDA channel and block it by a voltage-dependent mechanism.

Methods

Neuronal cultures

Neurons from the striatal, hippocampal and thalamic regions of Wistar rat foetuses at 18–20 days of gestation were dissociated and cultured as previously described (Alkondon & Albuquerque, 1993), and so were neurons from the retinae of embryonic day 8 white Leghorn chick embryos (do Nascimento & de Mello, 1985). The cells were plated on poly-lysine or collagen-coated plastic culture dishes, and were maintained in serum-supplemented medium, at 35°C and in 10% CO_2 (rat neurons) or at 37°C and in 5% CO_2 (chick neurons). Chick retinal neurons were used 1–3 weeks after plating, while rat neurons were used 2–5 weeks after plating.

Electrophysiology

Whole-cell and single-channel currents were recorded with standard patch-clamp techniques, using an Axopatch 200A unit and pCLAMP data acquisition software (Axon Instruments, Foster City, CA, U.S.A.). Experiments were done at room temperature (21–23°C). Whole-cell and single-channel currents were recorded with borosilicate glass microelectrodes of 1.5–3.5 MΩ or 7–10 MΩ, and low-pass filtered at 200 Hz or 3 kHz (−3 db, Bessel), respectively. Series resistance was always less than 20 MΩ under whole-cell clamp and was left uncompensated; currents larger than about 600 pA were excluded from the analysis to minimize voltage errors. For both chick and rat neurons, the same extracellular solution and Cs^+-based, F^--containing intracellular (pipette) solution were used. The composition of the extracellular solution was (in mM) NaCl 165, KCl 5, $CaCl_2$ 2, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulphonic acid] (HEPES) 5, D-glucose 10, NaOH 2, (pH 7.3, 340 mOsm kg^{-1}); while the intracellular solution was CsCl 80, CsF 80, ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (Cs^+ salt) 10, HEPES 10 (pH 7.3, 335 mOsm kg^{-1}). All test drugs were dissolved in extracellular solution with added ascorbic acid, either at 0.1% w v^{-1} or at 1 mol mol^{-1} of catecholamine, plus phenol red 0.005% w v^{-1}, and tetrodotoxin 0.3 μM, brought to pH 7.3 with NaOH. The solution bathing the cells was changed to a 'vehicle' solution containing ascorbic acid, phenol red, and tetrodotoxin a few seconds before each challenge with the test drugs. Except for the disappearance of most synaptic currents, no changes in the baseline current were seen when switching from the drug-free superfusion to the vehicle. The recording chamber consisted of the culture dish with a pierced silicone rubber insert sealed onto the bottom, leaving a 1.6 cm^2 rectangular area exposed. The bath volume was 250 μl, and was continuously superfused with drug-free extracellular solution, at 0.5–1.0 ml min^{-1}. Test solutions were applied to the cells by gravity, only one solution flowing at a time, using a motorized flow-pipe array system to switch solutions (Mayer *et al*., 1989). The array was made of thin-wall glass tubes (diameter: 400 μm), the lower rim of the active tube being positioned about 80 μm above and 300 μm away from the cell. Complete solution exchange over a whole-cell clamped neuron occurred with an exponential time constant of 10–20 ms. Highly reproducible results were achieved by briefly purging the solutions (away from the cells) just before each drug challenge, thus avoiding problems of dilution and photo-oxidation of the drugs at the tube ending. Two types of protocol were used. In one, the cells were exposed to dopamine for several seconds and then a pulse of NMDA plus dopamine was given, and the peak current was measured. In this condition, dopamine was presumably in equilibrium with the preparation before the evoked response and was at constant concentration throughout the drug challenge. The other protocol had three steps. The neuron was sequentially exposed to a 2-s pulse of NMDA solution, during which the current reached its peak and started to decay, then to a 2-s pulse of NMDA plus dopamine, then again to a 2-s pulse of NMDA alone, followed by the agonist-free, vehicle solution (see Figure 1b and c). The total duration of the NMDA pulse was kept at 6 s and the interval between pulses was at least 2 min to minimize rundown of the response. Because of receptor desensitization, even at the lowest NMDA concentration used, 10 μM, the whole-cell current usually did not reach an apparent steady-state during the total 6-s application of NMDA. The effect of dopamine was measured in the middle of the pulse (at 3 s) and expressed as the per cent amplitude of the current compared with the value expected in the absence of dopamine. For each drug challenge, this expected value was estimated by interpolation of the current's time course between the first 2 and last 1 second of the 6-s NMDA pulse. Thus, the decaying trend of the NMDA-evoked current was taken into account in the measurements, and the experimental condition approached that of a pulse of dopamine on a preparation equilibrated with NMDA.

Analogue data from whole-cell and single-channel recordings were sampled at 2 and 20 kHz, respectively, and were analysed with the pCLAMP programs (Axon Instruments). Mean single-channel open times were estimated from a single exponential fit to the frequency distributions, excluding events briefer than 0.4 ms. Unless otherwise noted, results are reported as mean ± s.e.mean of the values obtained from several cells, each value being the average of repeated measurements made in the same cell. The half-inhibitory concentration (IC_{50}) and Hill coefficient (n_H) were estimated by least-squares curve fitting, with the maximum response constrained to 100%.

Dopamine, (+) and (−)-SKF38393 (2,3,4,5,-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine), and R(+)-SCH23390 (7-chloro- 8- hydroxy- 3- methyl -1-phenyl- 2, 3, 4, 5-tetrahydro-1*H*-3-benzazepine) were supplied by Research Biochemicals International (Natick, MA, U.S.A.), most other drugs were from Sigma (St. Louis, MO, U.S.A.).

Results

NMDA-evoked currents

Whole-cell currents could be recorded in nearly all neurons tested in response to pulses of NMDA 10–50 μM with glycine 10 μM, at a membrane potential of −57 mV. The currents reached peak within less than 200 ms and then started to decay, showing desensitization. On average, the peak current amplitude was larger in neurons from the rat hippocampus

than in neurons from striatum, thalamus and chick retina. This difference was correlated with the larger cell size of the hippocampal neurons. The rate and extent of desensitization of the NMDA response were larger in striatal cells than in the other cell types. For this reason, the conventional protocol of peak amplitude measurement was preferred in striatal cells, while the three-step protocol was used with the others. Although the four preparations were not systematically compared, it was found that the dopaminergic agents had qualitatively similar effects in retinal, striatal, hippocampal and thalamic neurons. For convenience, most of the quantitative data was obtained from hippocampal neurons, which yielded more robust responses to NMDA.

Effect of dopamine on the NMDA response

The NMDA-gated currents were reduced in the presence of dopamine, when at concentrations above 50 μM. In striatal cells, responses to a 3-s pulse of NMDA 50 μM were evoked every 2 min, alternating controls with test responses in the presence of dopamine. To circumvent time-dependent changes in the response, the peak amplitude of each test response was compared with the average of the peak amplitudes of the two bracketing controls. The test response was then expressed as a percentage of the interpolated control. As shown in Figure 1a, in the presence of dopamine 100 μM the response was $87.7 \pm 1.7\%$ of control, while with dopamine 500 μM, the reduction was to $69.5 \pm 2.9\%$ (three cells). The differences between dopamine and control and between the two concentrations were significant with $P < 0.001$ (analysis of variance). Dopamine alone did not affect the baseline current (not shown).

The three-step protocol was then used to look for a fast modulatory effect of dopaminergic agents on the NMDA response, which would be consistent with the hypothesis that dopamine binds directly to the NMDA receptor-channel (do Nascimento *et al.*, 1998). Indeed, the inhibitory effect developed in less than 100 ms and subsided in less than 500 ms, as seen from the time courses of current decay and recovery at the beginning and the end of the dopamine pulse (Figure 1b and c). Based on responses recorded from rat hippocampal neurons (Figure 1b) and chick retinal neurons (Figure 1c), one could assume that the drug-receptor interaction was nearly at equilibrium at the middle of the 2-s dopamine pulse, where the effect was measured. For any concentration of dopamine tested (50–2000 μM), the per cent inhibitions observed in all four cell types were similar. Different concentrations of dopamine were applied in random order to hippocampal neurons, to obtain a concentration-response curve (Figure 2b). The concentration of dopamine producing half-maximum inhibition of the NMDA response (IC_{50}) was 1.2 mM (n_H: 1.16), with the membrane potential held at -57 mV.

Effect of (+)-SKF38393 on the NMDA response

To characterize the putative dopaminergic receptor involved in the effect of dopamine, we tested more selective drugs. (+)-SKF38393 was the chosen D_1 agonist, while its enantiomer, (−)-SKF38393, was used to test for stereospecificity of the effect. While (+)-SKF38393 is a potent D_1-type agonist in adenylate cyclase assays, showing an EC_{50} of the order of 10^{-8} M, the (−) enantiomer is nearly without effect (Kaiser *et al.*, 1982). (+)-SKF38393 blocked the NMDA-gated currents, and was found to be more potent than dopamine. At 50 μM, (+)-SKF38393 reduced the current to $47.3 \pm 3.0\%$ of control in 13 thalamic neurons. In hippocampal neurons, an IC_{50} of 58.9 μM (n_H: 1.21) was estimated from the concentration-response curve (Figure 2a and b).

In one retinal and three thalamic cells, both enantiomers were applied at 50 μM, but, while (+)-SKF38393 blocked the NMDA response, (−)-SKF38393 had little or no effect (Figure 3a and b). These results suggested that a D_1-like receptor was involved. To further test this hypothesis, the

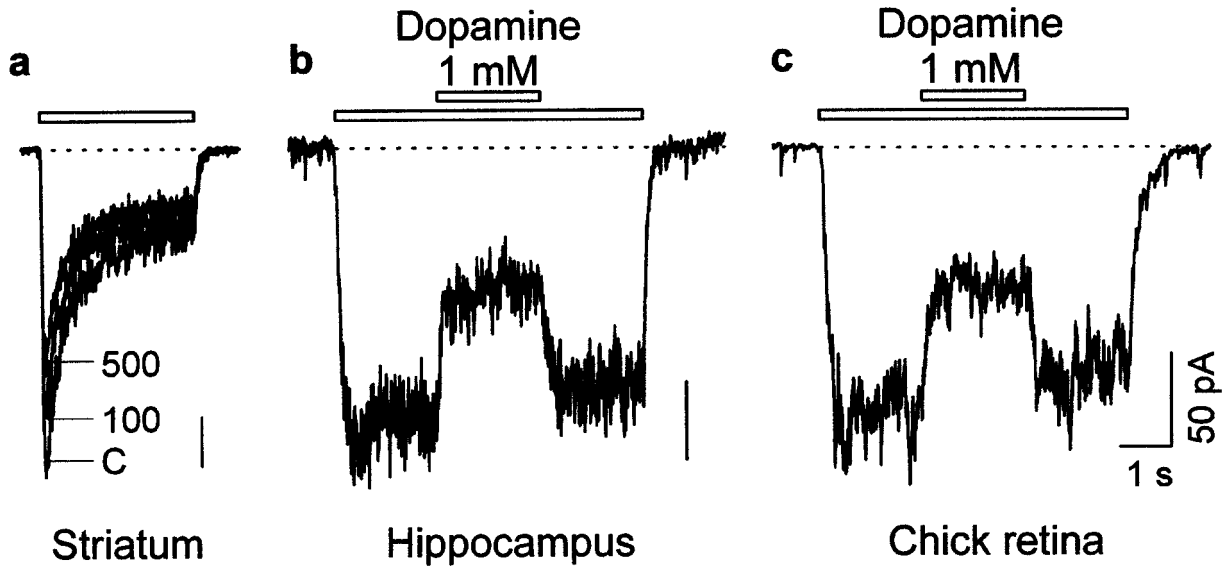

Figure 1 Inhibition by dopamine of NMDA-gated currents. (a) NMDA 10 μM plus glycine 10 μM applied in a 3-s pulse (open bar) to a striatal neuron clamped at -57 mV elicited an inward current that quickly decayed to about 25% of the peak amplitude. When the pulse was applied in the presence of dopamine 100 or 500 μM, the peak current amplitude was reduced. (b and c) A 6-s pulse of NMDA 10 μM applied to a rat hippocampal (b) or a chick retinal neuron (c) clamped at -57 mV elicited slowly-decaying inward currents that were reversibly reduced when dopamine 1 mM was co-applied for 2 s, in the middle of the NMDA pulse. The horizontal dashed line indicates the baseline current level and the long and short open bars on top of the traces indicate the durations of the agonist (NMDA) and antagonist (dopamine) pulses, respectively. The vertical calibration bars correspond to 50 pA in (a, b and c).

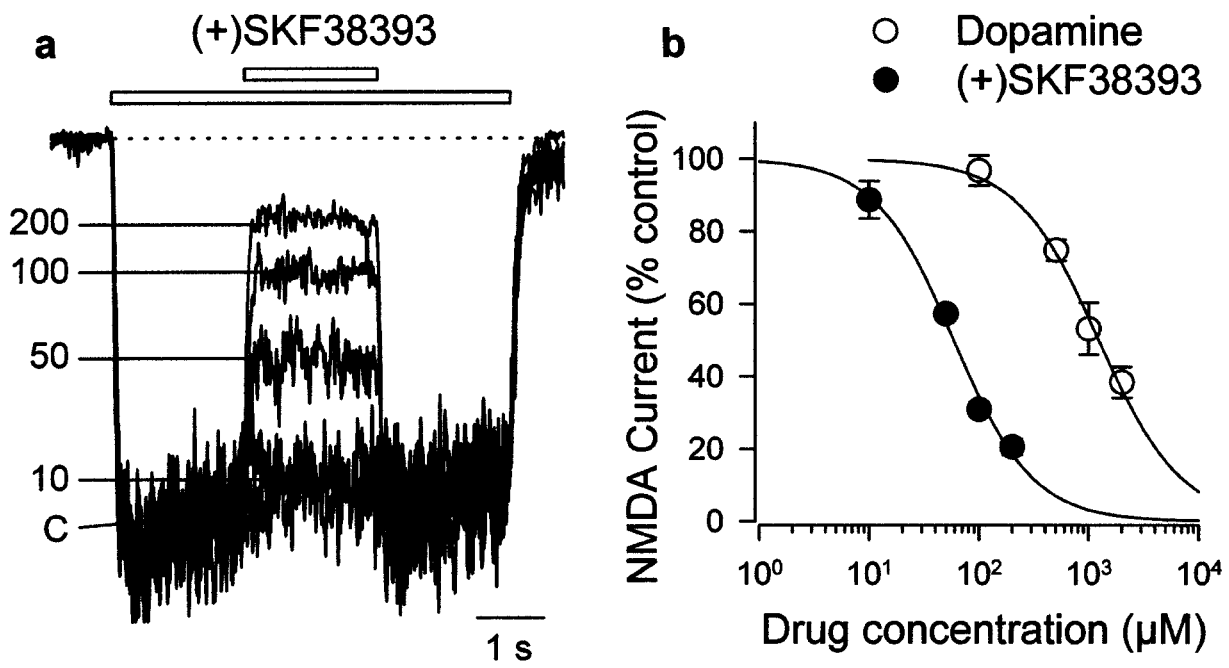

Figure 2 (a) Inhibition of currents elicited by NMDA 10 μM and glycine 10 μM by (+)-SKF38393. Five traces obtained from the same hippocampal neuron were scaled to the same peak amplitude and superimposed, illustrating the control and the effect of the indicated concentrations of (+)-SKF38393 in micromolar units. The peak amplitude of the NMDA current ranged from -100 to -150 pA in this cell. (b) Inhibition curve of the NMDA-gated currents by dopamine and (+)-SKF38393 in hippocampal neurons clamped at -57 mV. Each symbol and error bar mark the mean $\pm$ s.e.mean of 3–8 neurons. The best-fitting curves were obtained with IC_{50} and n_H values of 1.2 mM and 1.16 for dopamine, and of 58.9 μM and 1.21 for (+)-SKF38393.

experiments were repeated in the presence of R(+)-SCH23390, a specific D_1 receptor antagonist. The D_1 antagonist was added to both the vehicle and the NMDA-containing solutions and was introduced at least 1 min prior to the challenge with NMDA. In four thalamic neurons exposed to R(+)-SCH23390 10 μM, (+)-SKF38393 reduced the current to $49.8 \pm 5.0\%$, while in three retinal neurons the reduction was to $56.2 \pm 0.7\%$. These degrees of current blockade by (+)-SKF38393 50 μM were not significantly different from those obtained in the absence of R(+)-SCH23390. At these concentrations of agonist and antagonist, the binding of (+)-SKF38393 to D_1 receptor sites would be more than 95% inhibited (Andersen & Jansen, 1990). Therefore, (+)-SKF38393 seemed not to be acting through the classic D_1 receptor pathway.

The effect of (+)-SKF38393 was also examined on the response to a non-NMDA glutamatergic agonist, using the three-step protocol. (+)-SKF38393 (50 μM) did not measurably affect the currents activated by kainate 50 μM, but blocked NMDA-gated currents by about 50% in four thalamic cells (Figure 3c).

Voltage-dependence of the inhibitory effect

The degree of blockade of the NMDA currents by both dopamine and (+)-SKF38393 was tested at different transmembrane voltages. In striatal neurons held at -87 mV, dopamine 100 μM reduced the peak amplitude of the NMDA response to $77.3 \pm 3.6\%$ of control (three cells). With dopamine 500 μM, the reduction was to $45.1 \pm 1.0\%$ of the control values (three cells). These values were significantly lower ($P < 0.001$, analysis of variance) than those observed at -57 mV (c.f. Figure la).

The effect of voltage was further tested in hippocampal neurons using the three-step protocol. Figure 4 shows that the degree of blockade by dopamine 1 mM increased as the membrane potential changed from $+33$ mV to -87 mV. Similar results were obtained in the presence of a physiological concentration of Mg^{2+}, a voltage-dependent blocker of the NMDA channel. At -57 mV, dopamine 1 mM with $MgCl_2$ 1 mM reduced the current to 68.6% of control, and the blockade lessened as the membrane was depolarized. The effect of (+)-SKF38393 was also voltage-dependent; when this D_1 agonist was applied at 50 μM (in Mg^{2+}-free solutions), the NMDA-gated currents were reduced to $72.4 \pm 12.7\%$ at -37 mV and to $42.2 \pm 10.9\%$ at -97 mV (data are mean $\pm$ s.d. from four cells). Assuming that the inhibitory effect involved electrostatic interaction of the drugs with a single site of action, the plots of current fraction vs membrane potential were fitted with an exponential function. The Hill coefficients estimated from the concentration-response curves, 1.21 and 1.16, are compatible with this assumption of a single binding site. In the experiment with dopamine, the current was reduced e fold ($2.7\times$) when the membrane was hyperpolarized by 83 mV (Figure 4b), while in the four experiments with (+)-SKF38393, the equivalent change in average current occurred with a hyperpolarization of 78 mV (Figure 4d).

Effect of (+)-SKF38393 on NMDA-gated single-channel currents

The mechanism of the inhibitory effect of (+)-SKF38393 was further studied at the single-channel level. Outside-out membrane patches excised from hippocampal neurons were exposed to solutions containing NMDA 50 μM and glycine 10 μM, with and without (+)-SKF38393 50 μM, at -87 mV. The currents activated by NMDA appeared in bursts of events that could be classified in at least two distinct amplitude levels, the most prevalent having a mean amplitude of -3.7 pA. (+)-SKF38393 did not affect the mean current amplitude or the frequency of channel openings (not shown), but shortened the open times of the NMDA-gated channels (Figure 5). In two

cells, the mean open times were 1.81 and 2.43 ms in the control condition, and fell to 0.78 and 0.76 ms, respectively, in the presence of (+)-SKF38393. By inspection, the burst length also appeared shorter, but due to the high frequency of openings in these experiments, bursts could not be unambiguously defined for statistical analysis. The blockade was quickly reversed upon removal of (+)-SKF38393.

Discussion

Dopamine inhibited the ionic currents activated by NMDA in neurons from three different rat brain regions and from the chick retina. This result, obtained with electrophysiological methods, is in agreement with the observed inhibitory effect of dopamine on NMDA-induced GABA release described by do

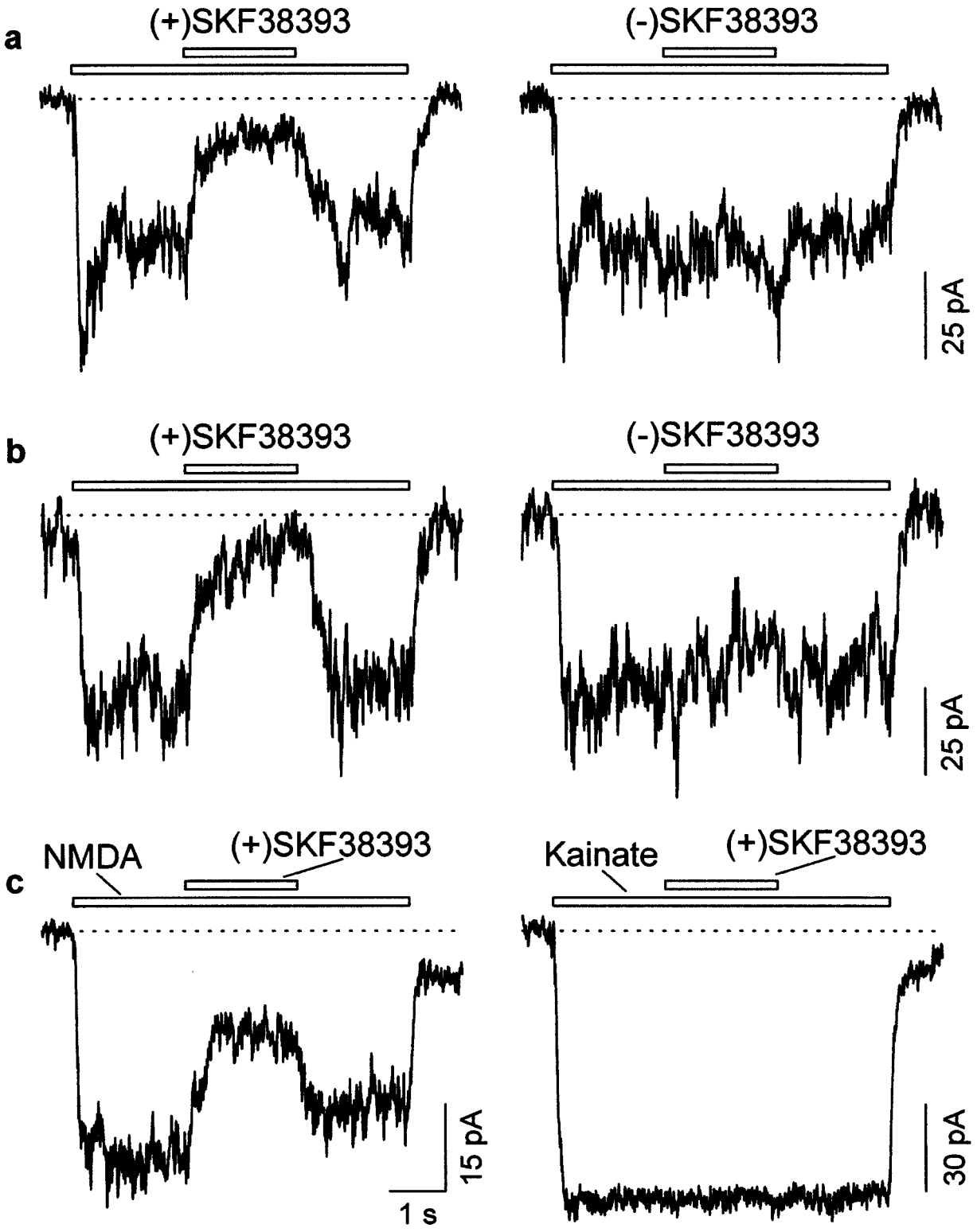

Figure 3 Stereoselectivity and specificity of the inhibition of the NMDA currents by (+)-SKF38393. Currents activated by NMDA 50 μM and glycine 10 μM were blocked by the (+), but not by the (−) isomer of SKF38393 (50 μM) when tested in the same neuron of the chick retina (a) or rat thalamus (b). In another thalamic neuron (c), (+)-SKF38393 50 μM reduced the NMDA-gated current to 48.5%, but the non-desensitizing current activated by kainate (50 μM) was unaffected.

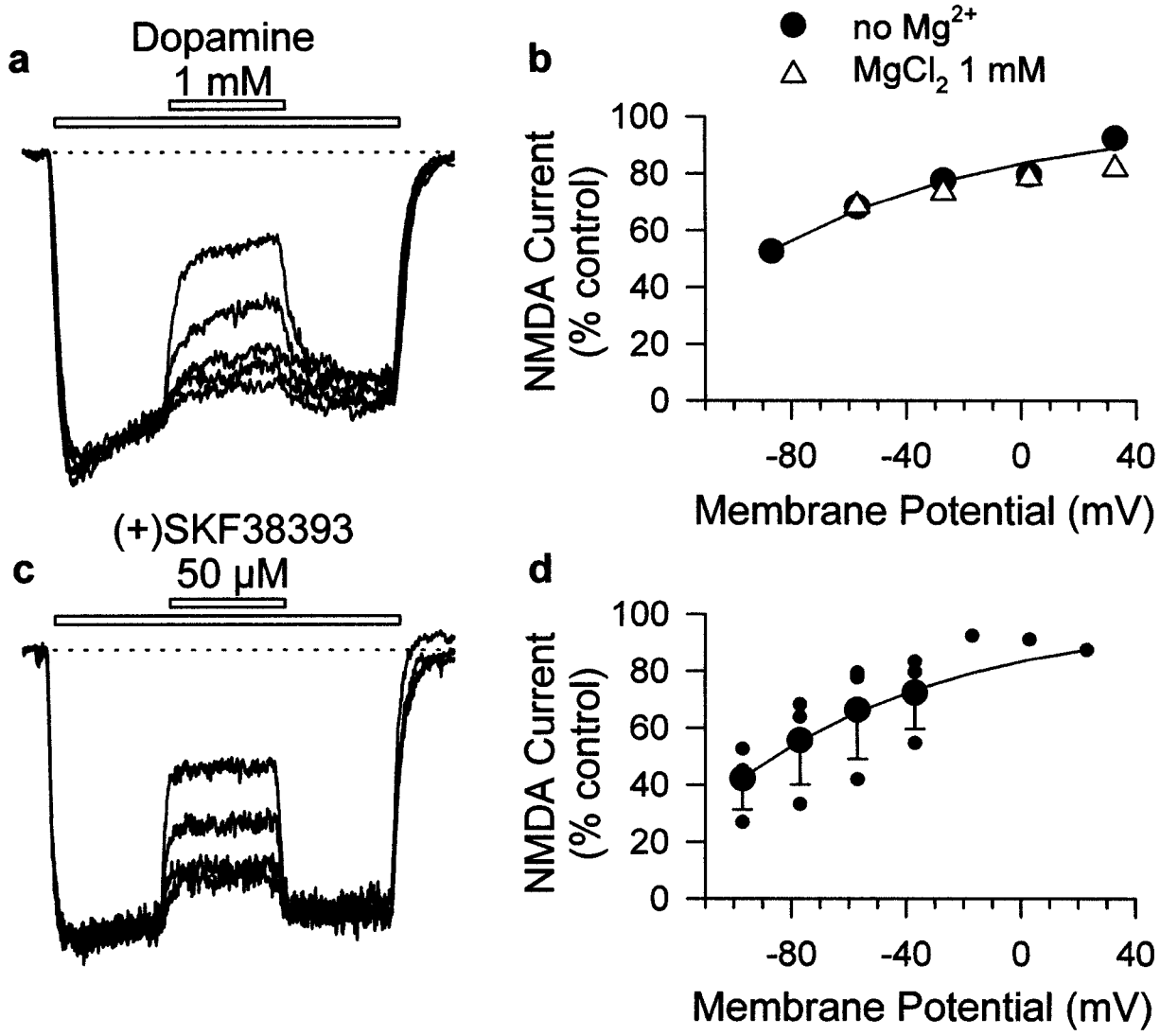

Figure 4 Voltage-dependence of the blocking effect in hippocampal neurons. (a) Currents activated by NMDA 10 μM and glycine 10 μM with a superimposed pulse of dopamine 1 mM in one hippocampal neuron held at +33, +3, −27, −57 and −87 mV were scaled and superimposed to show that the degree of current blockade increases with hyperpolarization. (b) Plots of NMDA current in dopamine vs membrane potential. The data points obtained in the absence of Mg^{2+} are from the experiment in (a), and are shown with the best-fitted exponential curve. The mean data points from two experiments done in the presence of $MgCl_2$ 1 mM also lie close to the curve. (c) Traces from another hippocampal neuron to which NMDA and (+)-SKF38393 50 μM were applied at −37, −57, −77, and −97 mV, scaled as in (a). (d) Plot of current in (+)-SKF38393 vs membrane potential (means±s.d.) from four cells, including that in (c). The data from individual cells and the best-fitting exponential are also shown.

Nascimento *et al.* (1998), and shows that the effect is not restricted to chick retinal neurons. Three key pharmacological features of the effect were similar to those reported in that study. First, the current-blocking effect of dopamine was mimicked by the D_1 agonist (+)-SKF38393, but was not prevented by the D_1 antagonist SCH23390. Second, the effect of (+)-SKF38393 was stereospecific, since the (−) enantiomer was nearly ineffective at 50 μM, close to the half-inhibitory concentration of (+)-SKF38393 (58.9 μM). Third, the inhibition was not a non-specific channel-blocking effect, because (+)-SKF38393 50 μM blocked the NMDA response by about 50% while not affecting the kainate response in the same neuron. Based on these similarities, it seems that blockade of ionic current through the NMDA receptor-channel underlies, at least in part, the inhibition by dopamine of glutamate-induced GABA release previously described in chick retinal neurons.

Our results provide support to the hypothesis that the inhibition is due to direct binding of the dopaminergic agonists to the NMDA receptor-channel, probably inside the channel pore. The three-step protocol unveiled a fast kinetics of blockade of the NMDA current by dopamine and (+)-SKF38393. A blocking rate of the order of tens of milliseconds was not to be expected if the effect were mediated by a metabotropic pathway, and in turn suggests that dopamine was binding directly to the NMDA receptor-channel macromolecule. Also, the degree of current blockade varied with transmembrane voltage, indicating that the blocking reaction occurred within the transmembrane electric field. This voltage dependence and the shortening of single-channel open times are characteristic features of many drugs that block open channels by binding inside the pore, with electrostatic forces contributing to the binding. The cationic nitrogen of dopamine and (+)-SKF38393 could confer the appropriate voltage dependence to the binding, since a more negative voltage would attract more drug to its binding site, causing more blockade as was indeed observed (see Figure 4). If dopamine and (+)-SKF38393 were binding inside the pore, they could

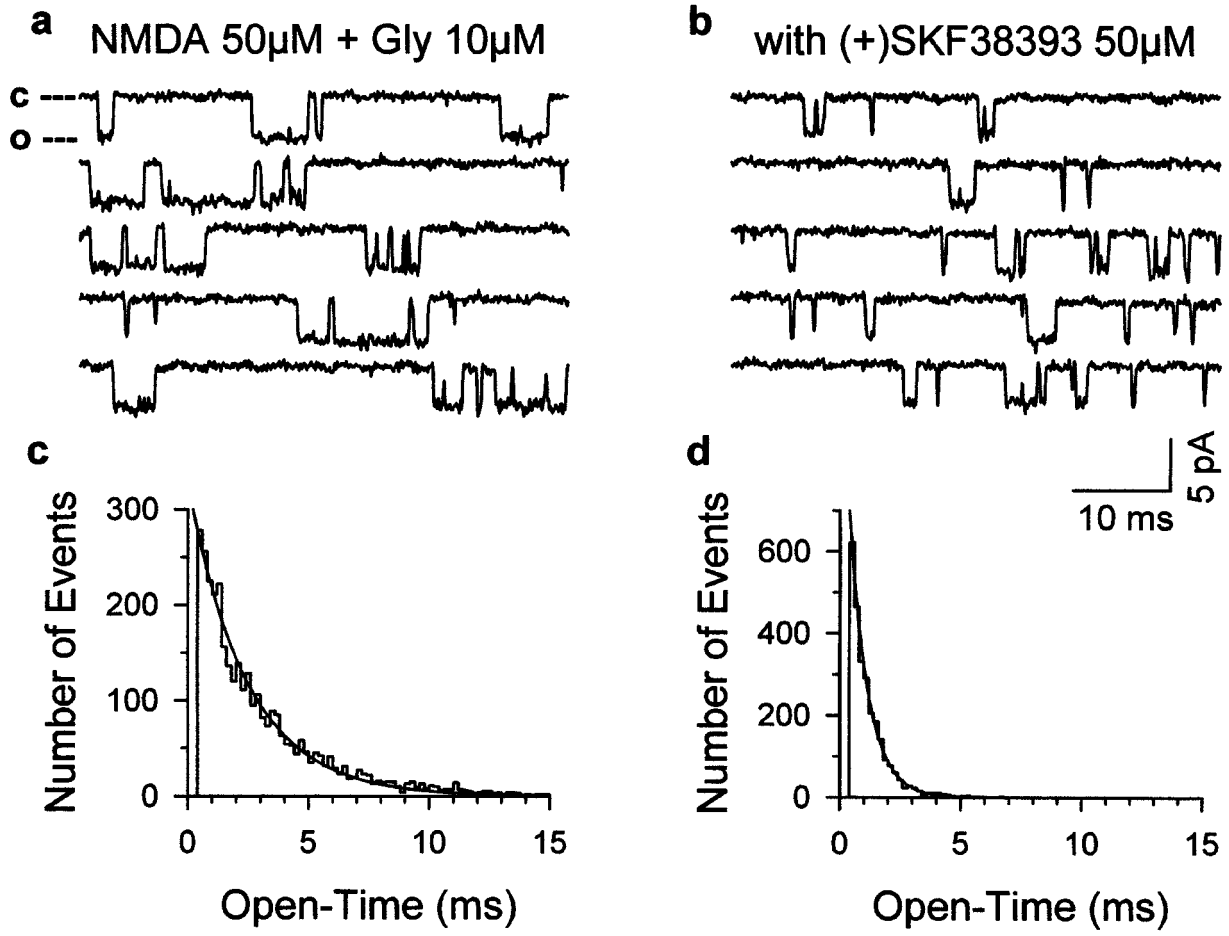

Figure 5 Effect of (+)-SKF38393 on channel open time in hippocampal neurons. Single-channel currents activated by NMDA 50 μM and glycine 10 μM in one outside-out membrane patch (a) appeared shorter when (+)-SKF38393 50 μM was added (b). Open-time histograms from these recordings could be fitted with simple exponential distribution functions. With NMDA and glycine alone (c), the mean open time was 2.43 ms, while in (+)-SKF38393 (d), it was 0.76 ms. The patch was held at -87 mV. Closed and open-channel current levels are labeled c and o, respectively, in panel (a).

perhaps interfere with the binding of other known pore ligands. Indeed, it was recently reported that (+)-SKF38393 100 μM inhibits the binding of dizocilpine (MK801) to synaptic membranes of rat prefrontal cortex (Gandolfi & Dall'Olio, 1996). However, the physiological NMDA receptor open-channel blocker, Mg^{2+}, did not appear to interfere markedly with the effect of dopamine when both were present at 1 mM, except perhaps for some reduction in the voltage dependence (Figure 4b). Based on inspection of the single-channel current traces, the channel-blocking mechanism of (+)-SKF38393 was apparently not the same as the fast-type described for Mg^{2+} (Ascher & Nowak, 1988). The bursts of openings seemed to include less events in the presence of (+)-SKF38393, and there was no evidence of extra unresolved brief closures. It is possible that the drug bound to the open channel with a relatively slow dissociation rate, inducing long-lived blocked states that could not be distinguished from other closed states in the receptor activation mechanism.

Among the many cells in which recordings were made at -57 mV, the degree of blockade seemed to be inversely correlated with the current amplitude and the whole-cell access resistance, i.e., with the voltage error. In the worst case included in the study, the theoretical voltage error would be 12 mV (0.6 nA $\times$ 20 MΩ). Given the voltage dependence of the inhibitory effect, such errors might have caused the potencies of dopamine and (+)-SKF38393 to be underestimated. An additional factor that could have reduced the measured blocking effect may have been an inadequate space-clamp, which would particularly affect NMDA channels located on dendrites. There, the membrane would be less polarized, and the NMDA channels less sensitive to blockade, than on the cell soma. This might explain why, on average, currents in retinal, striatal and thalamic cells were more sensitive to blockade than in hippocampal neurons, which were larger, had more prominent neurites and yielded larger currents. These and other factors probably contributed to the significant differences between the estimated IC_{50} in our electrophysiological experiments (1.2 mM) and in the assay of NMDA-induced GABA release in chick retinal neurons (60 μM; do Nascimento *et al*., 1998). There, NMDA causes release of GABA through a sodium and depolarization-dependent mechanism that could involve the activation of voltage-dependent channels (do Nascimento & de Mello, 1985; Hofmann & Möckel, 1991). It is possible that relatively small current reductions, as seen with dopamine 100 μM (e.g., 10–20% in striatal neurons), cause such significant changes in electrical activity of the retinal neurons that GABA release is disproportionately inhibited.

Even if dopamine were somewhat more potent than the dose-response relationship indicates, the concentrations at which it blocked NMDA-gated currents were high compared with the nanomolar concentrations required to induce its classical metabotropic effects. However, dopamine levels may reach a few hundred micromolar in the synaptic cleft (Dowling 1991) suggested that 'physiological concentrations are

<300 μM'). In the presence of 100 μM dopamine, the NMDA response in a hyperpolarized striatal neuron could be reduced by more than 20%, as shown above. Thus, the likelihood of a significant effect of endogenous dopamine on NMDA receptors *in vivo* depends on the proximity of these receptors to dopamine release sites. There is a high chance of interaction in the striatum, where presumed glutamatergic and dopaminergic terminals often make synaptic contact side-by-side onto the same dendritic spine (Freund *et al*., 1984). The interaction would also be more likely under the influence of dopamine uptake inhibitors. Therefore, it seems worth investigating if agents like cocaine can promote NMDA receptor blockade by synaptically released dopamine.

Dopamine receptor agonists showing NMDA receptor antagonism may be important in the context of therapy of Parkinson's disease. Several drugs effective in Parkinson's were shown to be NMDA receptor antagonists, including amantadine and memantine (Kornhuber *et al*., 1994; Lupp *et al*., 1992; Magazanik *et al*., 1996; Parsons *et al*., 1995; Stoof *et al*., 1992), budipine and biperiden (Jackisch *et al*., 1994). The inhibitory mechanism of amantadine and analogues on NMDA and acetylcholine-gated channels is similar to that of (+)-SKF38393 (Magazanik *et al*., 1996; Matsubayashi *et al*., 1997). Parsons and collaborators (Parsons *et al*., 1995) measured the onset and offset rates of NMDA channel blockade under whole-cell patch-clamp for amantadine, memantine and analogues, and correlated their dissociation constants with their relative antiakinetic potency. These authors suggested that a low affinity binding to the channel was a requirement for the antiakinetic effect. It is possible that the low-affinity blockade of NMDA receptors by (+)-SKF38393 is responsible for its small benefit compared to (+)-SKF82958 when both are associated with bromocriptine (Gossel *et al*., 1995), considering that the D_1-selective dopaminergic action alone is rather ineffective in Parkinson's or in other movement disorders (Braun *et al*., 1987; 1989).

In summary, we conclude that dopamine and (+)-SKF38393 can interact directly with the NMDA-subtype of ionotropic glutamate receptor, blocking the ion channel. The site involved with this effect is clearly distinct from those of the classical D_1 and D_2-type receptors, and might also be a target for endogenous dopamine. The benzazepine (+)-SKF38393 was more potent than dopamine in blocking the NMDA channel, and may become a prototype of novel drugs with both D_1-agonist and NMDA-antagonist effects.

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