Group I Metabotropic Glutamate Receptors Mediate an Inward Current in Rat Substantia Nigra Dopamine Neurons That Is Independent From Calcium Mobilization

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Guatteo, Ezia, Nicola B. Mercuri, Giorgio Bernardi, and Thomas Knöpfel. Group I metabotropic glutamate receptors mediate an inward current in rat substantia nigra dopamine neurons that is independent from calcium mobilization. J. Neurophysiol. 82: 1974–1981, 1999. Metabotropic glutamate receptors modulate neuronal excitability via a multitude of mechanisms, and they have been implicated in the pathogenesis of neurodegenerative processes. Here we investigated the responses mediated by group I metabotropic glutamate receptors (mGlurS) in dopamine neurons of the rat substantia nigra pars compacta, using whole cell patch-clamp recordings in combination with microfluorometric measurements of [Ca\(^{2+}\)] and [Na\(^+\)]. The selective group I mGlur agonist (S)-3,5-dihydroxyphenylglycine (3,5-DHPG) was bath-applied (20 \(\mu\)M, 30 s to 2 min) or applied locally by means of short-lasting (2–4 s) pressure pulses, delivered through an agonist-containing pipette positioned close to the cell body of the neuron. 3,5-DHPG evoked an inward current characterized by a transient and a sustained component, the latter of which was unaffected either by the superfusion of ionotropic excitatory amino acid antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-amino-5-phosphono-pentanoic acid (D-APV), nor by the sodium channel blocker tetrodotoxin (TTX). (S)-\(\alpha\)-methyl-4-carboxyphenylglycine (S-MCPG) and the more selective mGlur1 antagonist 7(hydroxyimino)cyclopenta[b]chromen-1a-carboxylate (CPCCOEt) depressed both 3,5-DHPG-induced inward current components and, although less effectively, the associated [Ca\(^{2+}\)] elevations. On repeated agonist applications the inward current characterized by a transient and a sustained component, the latter of which was uncovered only with long-lasting agonist applications. The fast component coincident with a transient elevation of [Ca\(^{2+}\)], whereas the total current was associated with a rise in [Na\(^+\)]. These responses were not affected either by the superfusion of ionotropic excitatory amino acid antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-amino-5-phosphono-pentanoic acid (D-APV), nor by the sodium channel blocker tetrodotoxin (TTX). (S)-\(\alpha\)-methyl-4-carboxyphenylglycine (S-MCPG) and the more selective mGlur1 antagonist 7(hydroxyimino)cyclopenta[b]chromen-1a-carboxylate (CPCCOEt) depressed both 3,5-DHPG-induced inward current components and, although less effectively, the associated [Ca\(^{2+}\)] elevations. On repeated agonist applications the inward current and the calcium transients both desensitized. The time constant of recovery from desensitization differed though less effectively, the associated [Ca\(^{2+}\)] elevations. On repeated agonist applications the inward current and the calcium transients both desensitized. The time constant of recovery from desensitization differed though less effectively, the associated [Ca\(^{2+}\)] elevations. On repeated agonist applications the inward current and the calcium transients both desensitized. The time constant of recovery from desensitization differed

INTRODUCTION

Electrophysiological studies both in vitro and in vivo have demonstrated that activation of metabotropic glutamate receptors (mGlurS) induces an excitation of dopamine neurons of the substantia nigra pars compacta (Meltzer et al. 1997; Mercuri et al. 1993). At present, eight different mGlur subtypes have been cloned, termed mGlurR1 through mGlurR8. The mGlur subtypes are classified into three groups, I through III, according to similarities in their primary structure, signal transduction pathways, and pharmacology, as derived from cloned receptors that were expressed in nonneuronal expression systems (Conn and Pin 1997; Houamed et al. 1993; Knöpfel et al. 1995; Masu et al. 1991; Nakanishi 1992). This multitude is further enhanced by the existence of splice variants of many of the mGlur subtypes. Alternative splicing of the mGlurR1 gene results in the expression of the splice variants mGlurR1a through mGlurR1d, which are differentially expressed at the cellular and at the subcellular level (Ferraguti et al. 1998; Grandes et al. 1994; Kosinski et al. 1998). Although dopamine cells do not exhibit high levels of immunoreactivity for the mGlurR1a splice variant (Testa et al. 1998), it has been more recently demonstrated that these cells predominantly express the mGlurR1b splice variant (Kosinski et al. 1998). Group I mGlurS expressed at the somatodendritic membrane are known to mediate an excitation and increased excitability in a variety of neuron types and by a multitude of mechanisms. These mechanisms include the depression of potassium conductances (Charpak et al. 1990), the activation of calcium-activated unselective cation conductances (Congar et al. 1997; Crepel et al. 1994; Raggenbass et al. 1997), the activation of calcium-independent unselective cation conductances (Guérineau et al. 1995), the up-regulation of calcium channel activity (Chavis et al. 1996), and the operation of an electrogenic Na\(^+\)/Ca\(^{2+}\)-exchange (Keele et al. 1997; Lee and Boden 1997; Staub et al. 1992). Furthermore, by virtue of their coupling to phosphoinositide hydrolysis and mobilization of calcium from intracellular stores, activation of group I mGlurS can result in the activation of calcium-sensitive potassium conductances (Fagni et al. 1991; Rainnie et al. 1994). Indeed, an inhibitory action mediated by mGlurR1d has recently been demonstrated in dopamine cells (Fiorillo and Williams 1998). With regard to the dopamine neurons of the substantia nigra pars compacta, mGlurS activation determines an inward current that is principally dependent on the extracellular concentration of sodium ions (Mercuri et al. 1993). Thus the purpose of the present study was to further characterize the mGlurR-mediated inward current in dopamine neurons and its possible relation to changes in [Ca\(^{2+}\)] and [Na\(^+\)]. A particular emphasis was given to the question whether there is a mechanistic relation...
ship between the excitation and the Ca$$^{2+}$$ signaling pathway that is expectedly activated by group I mGluRs.

METHODS

Electrophysiology

Preparation of rat midbrain slices was performed as described previously (Lacey et al. 1989; Mercure et al. 1995). In brief, horizontal slices including the substantia nigra and the ventral tegmental area were cut from the ventral mesencephalon of Wistar rats (150–250 g) anesthetized with halothane and killed. The brain was rapidly removed, and horizontal slices (thickness 300 μm) were cut by a vibratome starting from the ventral surface of the midbrain. A single slice was then transferred into a recording chamber and completely submerged in an artificial cerebrospinal fluid with continuously flowing (2.5 ml/min) solution at 35–36°C (pH 7.4). This solution contained (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl$$^2$$, 1.2 NaH$$^2$$PO$$^4$$, 2.4 CaCl$$^2$$, 10 glucose, and 18 NaHCO$$^3$$, gassed with 95% O$$^2$$-5% CO$$^2$$. The recording chamber was mounted on the stage of an upright microscope (Axioskop FS, Carl Zeiss) equipped for infrared video microscopy (Hamamatsu, Hamamatsu City, Japan) and video microfluorometry (ImproVision, Coventry, UK). Whole cell patch-clamp recordings were obtained from visually identified dopamine neurons (Bonci and Williams 1996) using pipettes made from boro-silicate glass (WPI 1.5 mm) and pulled with a PP 83 Narishige puller. The membrane voltage and current were acquired at 6- or 12-s intervals. Time courses of fluorescence values obtained at a given excitation wavelength were calculated over regions that included the cell bodies (“regions of interest,” defined as those pixels that exhibit at least 20–30% of maximal specific fluorescence) and were corrected for background fluorescence (measured from image regions free of dye fluorescence, ImproVision software). For measurements using fura-2 and SBFI the ratio of fluorescence obtained at excitation wavelengths of 340 and 380 nm were transformed into ion concentration using the method of Grynkiewicz (Grynkiewicz et al. 1985). The calibration of our system has been described previously (Guatteo et al. 1998; Knöpfel et al. 1998). Recordings of changes of [Ca$$^{2+}$$]$$^i$$, using calcium green are expressed as ∆F/F values as described earlier (Muri and Knöpfel 1994). Analysis and curve fitting was done using Origin 5 software (Microcal, Northampton, MA). Values in the text are expressed as means ± SE. Student’s t-test was used for statistical analysis.

Drug application

(S)-3,5-Dihydroxyphenylglycine (3,5-DHPG), (S)-α-methyl-4-carboxyphenylglycine (MCPG), 6-cyano-7-nitroquinoline-2,3-dione (CNPX), d–2-amino-5-phosphono-pentanoic acid (APV), and 7(hydroxyimino) cyclopenta[b]chromen-1a-carboxylate (CPCCOEt) were obtained from Tocris Cookson (Bristol, UK); tetrodotoxin (TTX) and thapsigargin were obtained from Calbiochem. All other compounds were from Sigma. Drugs were bath-applied by switching the solution to one containing known concentrations of drugs. An exchange of the solution in the recording chamber occurred in ~1 min. Agonists were also applied via a patch pipette that was positioned in close vicinity of the cell body and was connected to a pressure application system (Picospritzer, 20–30 psi, 0.1–10 s).

RESULTS

The database was derived from 54 visually and electrophysiologically identified principal neurons of the substantia nigra pars compacta. The criteria for the electrophysiological identification of dopamine neurons were, as described previously, a hyperpolarizing response to dopamine (30 μM) and a pronounced hyperpolarization-induced slowly relaxing inward current, I$$i$$ (Grace and Ond 1989; Lacey et al. 1989; Mercure et al. 1993, 1995; Yung et al. 1991). Cells were visually identified by their fusiform cell body and long unbranched proximal dendrites extending in the plane of the slice (cf. Fig. 1A) (Grace and Ond 1989).
Activation of group I metabotropic glutamate receptors induces an inward current associated with a rise in $[Ca^{2+}]_i$ and $[Na^+]_i$

About 5–10 min after formation of the whole cell configuration, the cell bodies and proximal dendrites of the dopamine neurons were loaded with the fluorescent dye contained in the patch pipette. Figure 1 shows recordings from a neuron loaded with the calcium indicator fura-2. Bath application of 20 $\mu$M 3,5-DHPG, a selective group I mGluR agonist (Schoepp et al. 1994), induced a biphasic inward current (Fig. 1B). The first component of the 3,5-DHPG-induced inward current had a mean amplitude of 142 ± 44 pA (mean ± SE, n = 8) and inactivated rapidly in the presence of the agonist uncovering a second more persistent component of 69 ± 13.2 pA. These agonist-induced currents were associated with an elevation in $[Ca^{2+}]_i$, of ~406 ± 106.8 nM (n = 4) when estimated with fura-2 (Fig. 1). Elevation in $[Ca^{2+}]_i$, of comparable size and decay time course were obtained when the cell was voltage clamped to −30 mV for 30 s. As illustrated in Fig. 1C, these calcium signals were relatively homogenous over the cell body and initial portion of the proximal dendrites, and time course analysis was restricted to this part of the neuron. Similar inward currents and $[Ca^{2+}]_i$ elevations were obtained with the less selective mGluR agonist 1S,3R-1-amino cyclopentane-1,3-dicarboxylate (1S,3R-ACPD) (70 $\mu$M, cf. Fig. 2C).

To determine whether the 3,5-DHPG–induced inward current was associated also with an elevation in $[Na^+]_i$, dopamine neurons were loaded with the Na$^+$-sensitive dye SBFI. These experiments revealed a large elevation in $[Na^+]_i$, during the mGluR-induced inward current (Fig. 2, A and C). Figure 2B illustrates $[Ca^{2+}]_i$, measurements using only calcium green in the pipette. This Ca$^{2+}$ dye indicates 3,5-DHPG–induced $[Ca^{2+}]_i$, changes with $\Delta F/F$ values of 0.76 ± 0.2 (n = 7) and whose time courses were consistent with those obtained with fura-2. To directly compare the time courses of the $[Ca^{2+}]_i$ and the $[Na^+]_i$, elevations with the components of the inward current in the same neuron, we combined SBFI with the indicator calcium green (Fig. 2C). When comparing the time course of the mGluR-induced $[Ca^{2+}]_i$, and $[Na^+]_i$, elevations, either within a single cell loaded with both dyes (Fig. 2C) or between cells (Figs. 1 and 2, A and B), it can be clearly seen that the $[Na^+]_i$ increase relates to the total inward current, whereas the $[Ca^{2+}]_i$ increase coincided with the initial transient component of the current. Similar differences in the agonist-induced time courses of $[Na^+]_i$, and $[Ca^{2+}]_i$, were observed in all 5/5 cells recorded with pipettes containing both SBFI and calcium green.

It has been recently reported that 3,5-DHPG can, under certain conditions, interact with N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors (Contractor et al. 1998). To demonstrate that, under the present conditions, 3,5-DHPG does not activate ionotropic receptors and does not cause opening of voltage-dependent dendritic channels to produce the inward current and to increase $[Na^+]_i$, we compared 3,5-DHPG-induced responses recorded in the presence of a cocktail of ionotropic antagonists (50 $\mu$M d-APV and 30 $\mu$M CNQX) and in the presence of TTX (1 $\mu$M) with responses recorded in the absence of these channel blockers (Fig. 3). The DHPG responses were not significantly affected either by the ionotropic glutamate receptor antagonists nor by TTX (ANOVA, P < 0.05). In the presence of 50 $\mu$M d-APV and 30 $\mu$M CNQX, the mean current amplitude amounted to 107 ± 6.1%, and the mean sodium increase to 111 ± 36.9% of controls (n = 6; Fig. 3A). In the presence of 1 $\mu$M TTX, the mean DHPG-induced current was 86 ± 6.2%, and the mean sodium increase was 100.6 ± 10.9% (n = 4) of control (Fig. 3B).

To isolate the initial transient component of the 3,5-DHPG–induced inward current, we applied the agonist via a pipette positioned close to the cell body and subjected to short-lasting (2 s) pressure pulses (see METHODS). Pressure ejection of the group I mGluR agonist induced short-lasting inward currents associated with $[Ca^{2+}]_i$, elevations comparable in size with those obtained with bath application of the agonist (Fig. 4A). These transient inward currents were also accompanied by elevations in $[Na^+]_i$, that, however, required averaging over
calcium elevations were depressed to a similar extent by 100 nM and are not affected by TTX.

3,5-DHPG and the corresponding increase in \([Na^+]_i\) under control conditions (left panels), in the presence of the ionotropic GluRs antagonists (middle panels), and in presence of TTX (right panels). Note that none of the channel blockers had any significant effect on the currents or on the sodium transients. B: histograms showing the mean magnitudes of inward currents and the increases of \([Na^+]_i\), induced by DHPG in the presence of TTX and the ionotropic antagonists cocktail. Note that the differences between the treated and untreated groups were not significant (P < 0.05).

several agonist puffs to be revealed with a reasonably good signal-to-noise ratio (Fig. 4B, n = 3). The size of these \([Na^+]_i\) elevations is nevertheless consistent with the level of \([Na^+]_i\), reached during generation of the initial transient inward currents in the experiments with bath application of the agonist (cf. Fig. 2A).

**MGluR subtype pharmacology**

3,5-DHPG is a specific agonist for group I mGluRs, and the responses described above are therefore most likely mediated by the mGlu receptor subtypes mGluR1 or mGluR5. To differentiate between these two subtypes, we took advantage of the fact that the compound CPCCOEt antagonizes responses mediated by mGluR1 much more potently than those mediated by mGluR5 (Casabona et al. 1997; Litschig et al. 1999). In addition, we compared the antagonism of the 3,5-DHPG–induced effects by CPCCOEt with that obtained with the broad-spectrum antagonist S-MCPG (Watkins and Collingridge 1994). CPCCOEt applied at a concentration of 100 μM and S-MCPG applied at 700 μM depressed the 3,5-DHPG–induced inward current to 40 ± 4.8% (n = 8) and 43 ± 7.7% (n = 8) of controls, respectively (Fig. 5). Likewise, the calcium elevations were depressed to a similar extent by 100 μM CPCCOEt and 700 μM S-MCPG (74.5 ± 9.9 and 72.3 ± 12.1% of controls, respectively, n = 8, Fig. 5).

**Desensitization of the fast transient inward current and \([Ca^{2+}]_i\) elevation**

When we used fast pressure application of 3,5-DHPG, we noted that the fast transient inward current, as well as the associated \([Ca^{2+}]_i\) elevations, exhibit rapid desensitization on repeated applications (Fig. 6). To determine the rate of recovery from desensitization, 2-s puffs of 3,5-DHPG were repetitively applied at varying time intervals to neurons loaded with calcium green. The time course of recovery from desensitization was determined by plotting the amplitude of the current and calcium green fluorescence, expressed as the fraction of a preceding undesensitized control response, against the time interval between the two applications (Fig. 6B). Both these functions could be reasonably well described by single time constants of 67.4 ± 4.4 s and 28.6 ± 2.7 s. These time constants, as well as the amount of recovery from desensitization at 15-, 30-, and 60-s intervals, differed significantly between these two mGluR1-induced responses, with the \([Ca^{2+}]_i\) signal showing a much faster recovery from desensitization. However, it might be possible that there is a nonlinear relationship between the dye response and free \([Ca^{2+}]_i\).

**Ca^{2+} signal results from mobilization of \([Ca^{2+}]_i\) ions from intracellular stores, and the inward current is resistant to depletion of intracellular stores and persists in the absence of \([Ca^{2+}]_i\) elevations**

The 3,5-DHPG–induced elevation of \([Ca^{2+}]_i\), could be due to an influx of \(Ca^{2+}\), for instance if the associated inward current was carried at least in part by \(Ca^{2+}\), or could result from a mobilization of \(Ca^{2+}\) from intracellular stores. To determine the source of the \([Ca^{2+}]_i\) elevations, responses induced by 3,5-DHPG were recorded first under control conditions and then after switching to a \(Ca^{2+}\)-free and 1 mM EGTA-containing extracellular solution. Influx of calcium during depolarization of the cell to −50 mV for 30 s was used to monitor the wash out of extracellular calcium. About 5 min after switching to the \(Ca^{2+}\)-free/EGTA extracellular solution, the first application of 3,5-DHPG induced only a reduced calcium response while subsequent agonist applications failed to elevate \([Ca^{2+}]_i\), as well as did depolarization of the membrane (Fig. 7). In the

[FIG. 3. Effects of DHPG are not antagonized by 6-cyano-7-nitroquinolxline-2,3-dione (CNQX) and N-2-amino-5-phosphono-pentanoic acid (D-APV) and are not affected by TTX. A: inward currents evoked by bath application of 3,5-DHPG and the corresponding increase in \([Na^+]_i\), under control conditions (left panels), in the presence of the ionotropic GluRs antagonists (middle panels), and in presence of TTX (right panels). Note that none of the channel blockers had any significant effect on the currents or on the sodium transients. B: histograms showing the mean magnitudes of inward currents and the increases of \([Na^+]_i\), induced by DHPG in the presence of TTX and the ionotropic antagonists cocktail. Note that the differences between the treated and untreated groups were not significant (P < 0.05).

FIG. 4. Pressure ejection of 3,5-DHPG in the close vicinity of the neuron.
absence of 1) extracellular calcium and 2) intracellular calcium transients, the agonist-induced inward currents persisted and amounted 177.1 ± 16.9% of controls (n = 6). After returning to control medium, 3,5-DHPG induced calcium elevations and inward currents that were not different from those obtained before washing out Ca^{2+}. As a complementary approach we also added thapsigargin to the extracellular solution to study the effect of abolition of the calcium elevations on the inward currents. After wash in of thapsigargin, 3,5-DHPG–induced calcium elevations were depressed, while the agonist induced inward currents persisted (139.0 ± 1.09% of controls, n = 2; Fig. 8A). Also in slices preincubated with thapsigargin (1 μM, 30–90 min) 3,5-DHPG–induced inward currents could still be recorded while the [Ca^{2+}]i elevations were reduced to 15 ± 7.4% of control (n = 4, Fig. 8B).

![Diagram](image)

**Fig. 5.** Antagonist pharmacology of the 3,5-DHPG–induced response of dopamine neurons. Inward currents (A) and calcium transients (B) induced by pressure ejection of 3,5-DHPG were recorded under control conditions (.), in the presence of 700 μM (S)-α-methyl-4-carboxyphenylglycine (MCPG; •) or 100 μM 7(hydroxyimino)cyclopropa[b]chromen-1a-carboxylate (CPCCOEt; ▲) in the bath, and after wash out of the antagonists (•). C: inward currents were depressed by 700 μM MCPG (43 ± 7.7%, of control, n = 8) and to a similar degree by 100 μM CPCCOEt (40 ± 4.8% of control, n = 8). D: calcium transients were less affected either by (S)-MCPG (72.3 ± 12.1% of control) or CPCCOEt (74.5 ± 9.9%, of control).

**Fig. 6.** Repetitive 3,5-DHPG applications reveal a desensitization of the responses. Inward currents elicited by pressure application of 3,5-DHPG were strongly reduced when the agonist was delivered repetitively at short time intervals (30–90 s). A: top trace shows the current trace during agonist applications (vertical arrows), at different time intervals. Bottom trace shows the corresponding calcium signals. Note that the currents exhibited a more pronounced and longer lasting desensitization than the calcium signals. B: the time course of recovery from desensitization was determined by plotting the amplitude of the current and the calcium green fluorescence, expressed as the fraction of a preceding undesensitized control response, against the time interval between the 2 applications. These time courses could reasonably be well described by functions of the form y = 100 – 100 e^{-t/τ} with single time constants, τ, of 67.4 ± 4.4 s (----) and 28.6 ± 2.7 s (-----) for the current and the calcium green fluorescence responses, respectively. These time constants, as well as the amount of recovery from desensitization at 30-, 60-, and 90-s intervals, differed significantly between these two responses, with the calcium green fluorescence signal showing a much faster recovery from desensitization.
DISCUSSION

In the present work we examined the relationship between the response mediated by mGluR1 and changes in [Ca\(^{2+}\)]\(_i\), and [Na\(^{+}\)], in dopamine neurons of the rat substantia nigra pars compacta. An inward current mediated by group I mGluRs that requires the presence of extracellular Na\(^{+}\) has been already described in these dopamine neurons (Mercuri et al. 1993) as well as in several other types of neurons (see INTRODUCTION). Here we characterized for the first time the accompanying changes in both [Ca\(^{2+}\)]\(_i\) and [Na\(^{+}\)], parameters that provide new and direct evidence to the mechanism underlying this current. In analogy with observations made in other types of neurons, it is conceivable that the inward current in dopamine neurons is mainly sodium dependent and mediated by a cation conductance with high selectivity for Na\(^{+}\) (Congar et al. 1997; Crepel et al. 1994; Guerineau et al. 1995; Raggenbass et al. 1997) or by an electrogenic Na\(^{+}\)/Ca\(^{2+}\) exchange (Lee and Boden 1997; Linden et al. 1994; Staub et al. 1992). The observation that the DHPG-induced inward current (see also Mercuri et al. 1993) and the increase in [Na\(^{+}\)], are not affected either by a cocktail of CNQX and D-APV nor by TTX, rule out the possibility that these responses are due to the activation of excitatory amino acid ionotropic receptors and/or the depolarization of unclamped dendrites. The fact that the inward current is associated with an increase in [Na\(^{+}\)], and neither requires a [Ca\(^{2+}\)]\(_i\) elevation nor the presence of calcium ions in the extracellular environment favors the involvement of a calcium-independent sodium-mediated conductance, rather than the activation of a calcium-activated cation channels or the sodium-calcium exchanger. As described previously (Mercuri et al. 1993), the mGluR-mediated current either caused a parallel inward shift of the current-to-voltage relationship, or had an extrapolated reversal potential above 0 mV. The parallel shift of the current-to-voltage relationship could be explained if, in addition to activation of a sodium conductance, a potassium conductance was changed during activation of mGluR1 in dopamine cells (Shen and Johnson 1997). Accordingly, we consistently observed that in the absence of [Ca\(^{2+}\)]\(_i\) elevations (e.g., in Ca\(^{2+}\)-free/EGTA or thapsigargin-containing extracellular solution; Figs. 7 and 8A), the 3,5-DHPG–induced inward current was larger than in control conditions. One explanation for these observations would be a contribution of a calcium-activated K\(^{+}\) conductance to the mGluR-induced conductance changes (Fiorillo and Williams 1998).

Group I mGluRs, which involves the subtypes mGluR1 and mGluR5, are known to couple to phosphoinositide hydrolysis and to induce mobilization of calcium from intracellular stores. Although this signal transduction pathway was primarily established in recombinant cells, it has also been identified in neuronal cells (Irving et al. 1992; Linden et al. 1994; Netzeband et al. 1997; Vranesic et al. 1991). Particularly, it has been suggested that mGluR5 couples more efficiently to phosphoinositide hydrolysis than does mGluR1 (Casabona et al. 1997). In agreement with the study mentioned above, we observed a rapid increase of [Ca\(^{2+}\)]\(_i\) during the activation group I mGluRs, which is associated with the fast phase of the inward current. The data obtained with the Ca\(^{2+}\)-free/EGTA or thapsigargin-containing extracellular solution definitely demonstrate that the elevation of intracellular calcium is due to mobilization of calcium from intracellular stores and that calcium is neither required as an activator nor as the main charge carrier for the inward current. Thus it appears that mGluR1 efficiently couples to an effector system that is independent from the phospholipase C/Ca\(^{2+}\)-signaling pathway in these cells.

The observation that CPCCOEt was more potent than R-MCPG in antagonizing the 3.5-DHPG–induced current is in accordance with a mGluR1-mediated effect (Batchelor et al. 1997; Litschig et al. 1999). Although both mGluRs antagonists were more effective in blocking the inward current than the signal transduction pathway associated with [Ca\(^{2+}\)]\(_i\), elevation, the pharmacological profile of [Ca\(^{2+}\)]\(_i\) elevation (more sensitivity to CPCCOEt than to R-MCPG) also suggests a mediation by mGluR1 rather than mGluR5. These findings are consistent with the notion that mGluR1 may be involved in the regulation of neuronal excitability in vivo.
with the prominent expression of mGluR1 in dopamine cells (Kosinski et al. 1998; Testa et al. 1994).

The 3,5-DHPG–induced inward current as well as the associated increase in [Ca$^{2+}$], exhibited a marked reduction on repeated applications that was rapid in onset and recovery. Responses mediated by mGluR5 desensitize due to receptor autophosphorylation, but this phenomenon appears to be absent in mGluR1 (Kawabata et al. 1996). The fact that the [Ca$^{2+}$]$_i$ responses were reduced after repeated agonist applications at short intervals can be sufficiently explained by a depletion of intracellular Ca$^{2+}$ stores. The rapid recovery from the ability to produce full-size [Ca$^{2+}$]$_i$ signals would then correspond to a rapid refilling of the intracellular Ca$^{2+}$ stores. Most of the properties of the 3,5-DHPG–induced inward current in dopamine cells are reminiscent of the cationic current described in hippocampal CA3 pyramidial cells (Guérineau et al. 1995). In fact, this current appears to be activated by metabotropic receptors via calcium-independent transduction process and shows desensitization with a complete recovery from desensitization within a few minutes.

Recently, it has been described that a tetanic stimulation induces slow mGluR-mediated excitatory or inhibitory synaptic potentials in dopamine neurons (Fiorillo and Williams 1998; Shen and Johnson 1997). Thus it is conceivable that the inward current described here might be the one generating the slow excitatory synaptic potentials.

**Physiological implications**

The scenario caused by the activation of group I mGluRs in the ventral mesencephalon is certainly complex and appears to operate at presynaptic and postsynaptic levels on the dopaminergic neurons. This comprehends the inhibitory response described by Fiorillo and Williams (1998), the excitatory response described by Mercuri et al. (1993) and Shen and Johnson (1997), and the presynaptic inhibition of transmitter release described by Bonci et al. (1997) and Wigmore and Lacey (1998).

Thus it might be speculated that discrete glutamatergic inputs to distinct zones of the dopamine cells might induce an inhibitory metabotropic response while more diffuse inputs driven at a higher frequency might mainly induce an excitatory metabotropic response. This phenomenon would ultimately change the functional significance of the synaptic inputs to the dopamine cells and consequently the state of their output in the terminal fields.

In a pathological context a dysfunction of the mGluR1-mediated inward current might alter the activity of the dopaminergic system in psychiatric disorders and participate to the excitotoxic neurodegeneration underlying Parkinson’s disease (Albin and Greenamyre 1992;Carlsson and Carlsson 1990).

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