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European Journal of Pharmacology 430 (2001) 167–173

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High-affinity inhibition of glutamate release from corticostriatal synapses by ω-agatoxin TK

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Received 15 February 2001; received in revised form 6 September 2001; accepted 7 September 2001

Abstract

To know which Ca^{2+} channel type is the most important for neurotransmitter release at corticostriatal synapses of the rat, we tested Ca^{2+} channel antagonists on the paired pulse ratio. ω-Agatoxin TK was the most effective Ca^{2+} channel antagonist (IC_{50} = 127 nM; maximal effect = 211% (with > 1 μM) and Hill coefficient = 1.2), suggesting a single site of action and a Q-type channel profile. Corresponding parameters for Cd^{2+} were 13 μM, 178% and 1.2. The *block* of L-type Ca^{2+} channels had little impact on transmission, but we also tested *facilitation* of L-type Ca^{2+} channels. The L-type Ca^{2+} channel agonist, *s*-(−)-1,4 dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyridine carboxylic acid methyl ester (Bay K 8644 (5 μM)), produced a 45% reduction of the paired pulse ratio, suggesting that even if L-type channels do not participate in the release process, they may participate in its modulation.

Keywords: ω-Agatoxin TK; ω-Conotoxin GVIA; Synaptic transmission; Neostriatum; Ca^{2+} channel

1. Introduction

Changes in the paired pulse ratio obtained from a pair of orthodromic responses mainly reflect the non-linearity of the presynaptic processes of transmission (Zucker, 1989, 1999). Postsynaptic processes do not change the paired pulse ratio under most circumstances but change both orthodromic responses in the same proportion (Dunwiddie and Hass, 1985). A postsynaptic process also changes the antidromic response. Consequently, the paired pulse ratio associated with field population spike recordings is a reliable protocol that can be used to pharmacologically screen receptors located at presynaptic nerve terminals (e.g., Creager et al., 1980; Dunwiddie and Hass, 1985; Malenka and Kocsis, 1988; Kahle and Cotman, 1993; Lovinger et al., 1993; Wheeler et al., 1994; Ameri et al., 1999; Barral et al., 1999, 2000). The results obtained with this protocol have been corroborated with other techniques such as intracellular- and whole-cell recordings (Nisenbaum et al., 1992; Lovinger et al., 1993; Brundege and Dunwiddie, 1996; Hernández-Echeagaray et al., 1998; Sullivan, 1999; Gerdeman and Lovinger, 2001).

In this work, the paired pulse ratio was used to test Ca^{2+} channel-targeting ligands whose actions are known to be located at corticostriatal terminals (Turner et al., 1993; Lovinger et al., 1994; Bargas et al., 1998; Hill and Brotchie, 1999). A main goal was to perform a concentration–response analysis for ω-agatoxin TK in corticostriatal terminals. Pharmacological analyses of Ca^{2+} channel antagonists based on the paired pulse ratio coincided with previous results obtained with different techniques and other central neurons (Takahashi and Momiyama, 1993; Wheeler et al., 1994; Teramoto et al., 1995, 1997; Reid et al., 1998; Iwasaki et al., 2000; Turner et al., 1993; Luebke et al., 1993; Dooley et al., 2000).

There is consensus about the importance of P/Q-type Ca^{2+} channels (α_{1A}) in the release process. We performed a concentration–response analysis of ω-agatoxin TK to ascertain whether the profile obtained is more similar to the P-type or the Q-type channel-blocking profile. Recent evidence has suggested a strong convergence of presynaptic modulators at Q-type Ca^{2+} channels (Wheeler et al.,

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01388-7

1994; Barral et al., 1999, 2000). ω-Agatoxin was compared with Cd^{2+}.

The Ca^{2+} entry involved in glutamate release at central synaptic terminals takes place through non-L-type Ca^{2+} channels (P/Q, N and R or α_{1A}, α_{1B} and α_{1E}) (Wu and Saggau, 1997; Randall and Benham, 1999). This is the same in the neostriatum, where the block of L-type Ca^{2+} channels does not affect corticostriatal transmission (Turner et al., 1993; Lovinger et al., 1994; Bargas et al., 1998; Hill and Brotchie, 1999). Nevertheless, what happens if L-type Ca^{2+} channels are *facilitated* instead of *blocked*? This point has not been sufficiently explored. We show here that the paired pulse ratio can give a preliminary answer to this question.

2. Methods

In our institution, animal treatment adheres to the European Community guidelines for the use of experimental animals. Dorsal neostriatal brain slices (400 μm) were obtained from anaesthetized male Wistar rats (100–120 g) as previously described (Bargas et al., 1998). Briefly, sagittal neostriatal slices were cut on a vibratome and incubated at room temperature in saline containing (in mM): 125 NaCl, 3 KCl, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 11 glucose, 0.2 thiourea and 0.2 (−)ascorbic acid (saturated with 95% O_2 and 5% CO_2; 298 mosM/l, pH = 7.4). Individual slices were transferred to a 5-mm^3 submerged chamber at 33–35 °C and superfused at 2–3 ml/min. Stimulation of cortical white matter or corpus callosum with concentric bipolar electrodes (50 μm in diameter) was used to elicit an orthodromic population spike (N_2) in the neostriatum (Cordingley and Weight, 1986; Malenka and Kocsis, 1988; Lovinger et al., 1993; Bargas et al., 1998). In some cases, a smaller antidromic (N_1) response could also be elicited (Cordingley and Weight, 1986; Bargas et al., 1998), probably due to current diffusion into the striatal tissue. Field population spikes, a composite of both field excitatory postsynaptic potential and population action potentials, were recorded with micropipettes filled with 0.9% NaCl and amplified with an AC amplifier. Records were digitized and stored on VHS tapes at 40 kHz and analysed off-line. Illustrations are the average of at least five records. Stimuli were brief square-voltage pulses (1–40 V; 0.1–0.4 ms; 0.06–0.2 Hz) delivered with a stimulator. Interstimulus time interval for paired stimuli was 20–60 ms. Peptidic toxins were tested in the presence of 10 μM bicuculline to eliminate the inhibitory $GABA_A$ receptor component (Bargas et al., 1998).

Two stimuli of equal strength were applied to determine the paired pulse ratio: S_2/S_1, where S_2 is the amplitude of the second and S_1 is the amplitude of the first orthodromic response (Fig. 1A,C). Stimulus strength was adjusted so

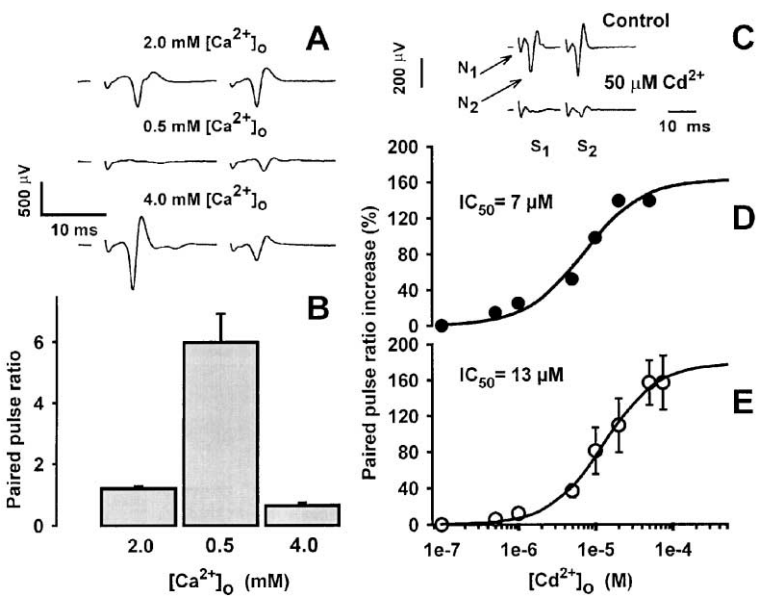

Fig. 1. The paired pulse ratio. (A) Top trace shows a pair of population spikes evoked with the same stimulus strength in saline with 2 mM external Ca^{2+}. Paired pulse ratio ≈ 1. Middle trace shows responses after external Ca^{2+} was decreased to 0.5 mM. There was paired pulse facilitation. The amplitude of both responses decreased. Bottom trace shows responses in the presence of 4.0 mM external Ca^{2+}. There was paired pulse depression. The first response was larger. (B) A summary histogram of experiments as in A yielded paired pulse ratios of (median and range) 1.1 (1.1–1.5; $n = 8$) for the control, 7.1 (2.1–8.9; $n = 7$) for low external Ca^{2+} ($P < 0.001$; Mann–Whitney *U*-test), and 0.63 (0.2–1.1; $n = 8$) for high external Ca^{2+} ($P < 0.003$; Mann–Whitney *U*-test). A one-way Kruskal–Wallis analysis of variance involving all three samples yielded $P < 0.001$ (Kruskal–Wallis statistic = 18.2). (C) The control shows a pair of orthodromic population spikes (N_2) with a paired pulse ratio ≈ 1. Antidromic population spikes were also present (N_1). External Cd^{2+} (50 μM) reduced both orthodromic population spikes and enhanced the paired pulse ratio. Antidromic population spikes did not change during Cd^{2+}. (D) A representative concentration–response plot for a single brain slice: $IC_{50} = 7.1$ μM; maximal response = 163% and nH = 1.15. (E) A summary concentration–response plot including four slices as in D yielded $IC_{50} = 12.8 \pm 4$ μM, maximal response = 179 ± 12% and nH = 1.25 ± 0.18.

that the paired pulse ratio was near 1.0 in the control recordings. The stimulus strength to achieve a paired pulse ratio near 1.0 was equal to the strength necessary to obtain about one-third of the maximal population spike amplitude. In a set of control experiments, the paired pulse ratio was mean ± S.E.M. = 1.2 ± 0.05, median = 1.1, range = 0.94–1.89, $n = 31$. Both the paired pulse ratio and its change as a percentage are reported. Concentration–response relationships were generated with percent changes in the paired pulse ratio produced by Ca^{2+} channel-targeting ligands (Barral et al., 1999, 2000). The range of responses for concentration–response analysis varied depending on the initial conditions, i.e., a control paired pulse ratio less than 1.0 would increase this range. However, we chose to have a control paired pulse ratio similar to 1.0 since the relatively weak stimulus strength used for these initial conditions allows a viable preparation that can be used for a longer time and the time needed by the antagonist to reach a stable block is shorter. We measured the paired pulse ratio once it achieved a stable value after antagonist application, i.e., when most probably the antagonist concentration had reached equilibrium with its bound form. This could take about 40 min for the lower concentrations. The dose–response relation was fitted with a non-linear Marquardt algorithm to the Hill equation:

$$\%\text{Paired pulse ratio} = \%\text{maximal paired pulse ratio} / \left(1 + \left(IC_{50}/[\text{ligand}]\right)^{nH}\right)$$

where IC_{50} is the half-maximal effect, [ligand] is the concentration of Cd^{2+} or ω-agatoxin TK (Figs. 1 and 3), and "nH" is the slope factor of the concentration–response function, i.e., an approximation to the Hill coefficient. Parameters were fitted using commercial software (Sigma Plot, Jandel, San Rafael, CA). The term IC_{50} ("inhibitory concentration" 50) is used for half-maximal effect, even when the concentration–response plots reported are ascending functions, since the increase in the paired pulse ratio is a measure of presynaptic inhibition or a decrease in the probability of transmitter release.

2.1. Drugs

$CdCl_2$, bicuculline (Sigma, St. Louis, MO), ω-conotoxin GVIA, ω-agatoxin TK (Peptides International, Louisville, KY or Alomone, Jerusalem, Israel); nitrendipine and *s*-(−)-1,4 dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyridine carboxylic acid methyl ester (*s*-(−)-Bay K 8644) (RBI, Natick, MA) were all added from freshly prepared stock solutions to the bath solution.

2.2. Data analysis

Non-parametric (Mann–Whitney *U*-test or Wilcoxon's *T*-test) statistical tests were used to compare sample data. $P < 0.05$ indicates statistical significance. Mean and standard error of the mean (S.E.M.) are given throughout the text as well as medians and ranges of each sample. The statistical test used is indicated in each case.

3. Results

3.1. Changes in external Ca^{2+} concentration

Fig. 1A top ($[Ca^{2+}]_O = 2.0$ mM) illustrates two consecutive orthodromic population spikes evoked by a pair of stimuli of equal strength adjusted to have a paired pulse ratio of about mean ± S.E.M. = 1.2 ± 0.07, median 1.1, range = 1.1–1.5, $n = 8$. Stimulus strength was not changed when the external Ca^{2+} concentration became 0.5 mM (Fig. 1A middle) in order to decrease Ca^{2+} entry to the terminals and to lower the release probability. After lowering $[Ca^{2+}]_O$, both responses decreased although the second one decreased less. Consequently, the paired pulse ratio increased in low Ca^{2+} to 6 ± 0.9, median = 7.1, range = 2.1–8.9, $n = 7$ ($P < 0.001$; Mann–Whitney *U*-test) (e.g., Katz and Miledi, 1968; Dunwiddie and Hass, 1985; Zucker, 1989, 1999; Gerdeman and Lovinger, 2001). This represents an increase of about 500% above the control. In contrast, raising the external Ca^{2+} concentration to 4.0 mM, to increase Ca^{2+} entry into the terminals and to enhance the release probability, produced the opposite effect (Fig. 1A bottom): Paired pulse ratio mean ± S.E.M. was 0.63 ± 0.1, median = 0.63, range = 0.2–1.1, $n = 8$ ($P < 0.003$; Mann–Whitney *U*-test). This represents a decrease of about 37% below the control. These changes could be produced consecutively, in the same preparation, and, therefore, were reversible. A one-way Kruskal–Wallis analysis of variance involving all three samples (low, normal, and high external Ca^{2+}) was highly significant ($P < 0.001$; Kruskal–Wallis statistic = 18.2). If the changes in external Ca^{2+} were explained by postsynaptic actions, both S_2 and S_1 responses would have changed in the same proportion. Fig. 1B shows a histogram summarizing the data. These results support the use of the paired pulse ratio in association with population spike recordings for evaluating presynaptic modulation in the corticostriatal preparation.

3.2. Cadmium ions

Fig. 1C illustrates the effect of external Cd^{2+} (50 μM) on the paired pulse ratio. As in the presence of low external Ca^{2+}, the paired pulse ratio became larger in the presence of Cd^{2+} due to the block of Ca^{2+} entry into the synaptic terminals. Note, however, that antidromic responses (N_1) were unchanged, supporting the presynaptic action of Cd^{2+} ions. The action of Cd^{2+} was concentra-

tion dependent. A concentration–response plot of the percent change of the paired pulse ratio measured in a single slice is illustrated in Fig. 1D ($IC_{50} = 7.1$ μM, maximal response = 163% and Hill coefficient = 1.15). Four experiments with slices from different animals were averaged for the summary plot in Fig. 1E (mean ± S.E.M.): $IC_{50} = 12.8 \pm 4$ μM, maximal response = 179 ± 12% and nH = 1.25 ± 0.18. This suggests that Cd^{2+} does not distinguish between the different types of Ca^{2+} channels involved in neurotransmitter release.

3.3. Peptidic Ca^{2+} channel antagonists

Both N- and P/Q-type Ca^{2+} channels are suggested to be involved in glutamate release at corticostriatal terminals (Hill and Brotchie, 1999; Bargas et al., 1998; Lovinger et al., 1994; Turner et al., 1993). In order to get an idea of the relative importance of these different channels, we performed the experiment illustrated in Fig. 2. The paired pulse ratio was enhanced in the presence of both ω-conotoxin GVIA (1 μM) and ω-agatoxin TK (400 nM), antagonists of N- and P/Q-type Ca^{2+} channels, respectively. These results suggest that both peptides act by reducing Ca^{2+} entry into the terminals and decreasing the release probability (Wheeler et al., 1994). ω-Conotoxin GVIA (1 μM) enhanced the paired pulse ratio from a mean ± S.E.M. = 1.25 ± 0.11, median = 1.07, range = 0.9–1.8, in the controls, to a mean ± S.E.M. = 2.3 ± 0.3, median = 2.01, range = 1.3–4.3, in the presence of the peptide ($n = 11$; $P < 0.01$, Wilcoxon's *T*-test). ω-Agatoxin TK (400 nM) enhanced the paired pulse ratio from a mean ± S.E.M. = 1.09 ± 0.05, median = 1.04, range = 0.9–1.2, in the controls, to a mean ± S.E.M. = 2.9 ± 0.3; median = 2.7, range = 2.2–4.0, in the presence of the peptide ($n = 5$; $P < 0.05$, Wilcoxon's *T*-test). Thus, as at other glutamate synapses, ω-conotoxin GVIA had less effect than ω-agatoxin TK: 88% vs. 159% change in the paired pulse ratio, respectively (taken from medians; $P < 0.02$; Mann–Whitney *U*-test), even though the conotoxin concentration was higher. Hence, a concentration–response analysis was done for the most potent ligand (ω-agatoxin TK; Fig. 3A) to see if the profile included both P- and Q-type channels, or only one of the two. It included a Lineweaver–Burk analysis (Fig. 3B) to determine the maximal effect (*y*-intercept in Fig. 3B), which was 211% at concentrations higher than 1 μM ω-agatoxin TK (see Fig. 3A). These concentrations were not used because agatoxins may not be specific at these concentrations (Sidach and Mintz, 2000). The non-linear fit (see Methods) of the concentration–response plot (Fig. 3A), using the maximal response obtained from the Lineweaver–Burk analysis as a constraint, yielded an $IC_{50} = 127 \pm 18$ nM. The Marquardt fit of the Hill equation (see Methods) for 24 different slices and experiments yielded a Hill coefficient of 1.2, which was not significantly different from 1.0 and similar to that obtained with Cd^{2+}, suggesting that the peptide mostly affects one site at the terminals. These results are very similar to those obtained

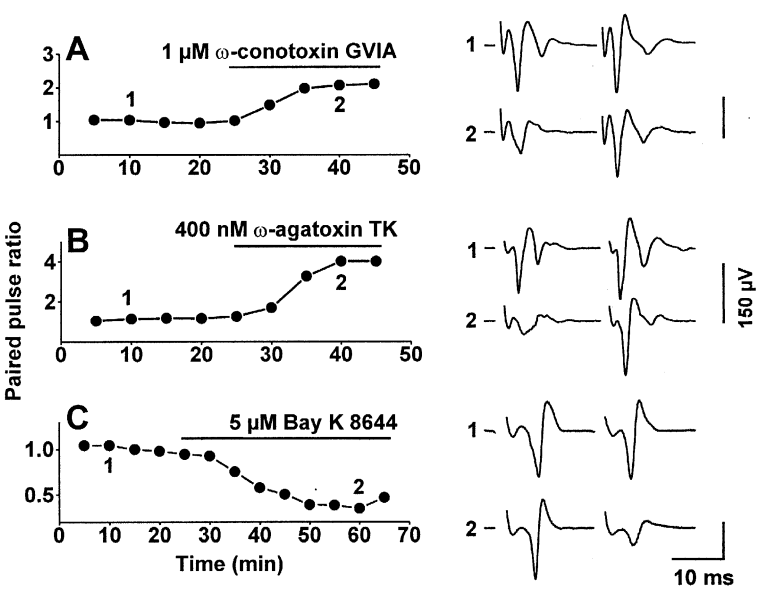

Fig. 2. Actions of Ca^{2+} channel-targeting ligands on the paired pulse ratio. (A) Time course of the paired pulse ratio before and during bath superfusion of 1 μM ω-conotoxin GVIA. The paired pulse ratio was increased during ω-conotoxin GVIA from (median and range) 1.07 (0.9–1.2) in the control to 2.01 (1.3–4.3) in the presence of the peptide ($n = 11$; $P < 0.01$, Wilcoxon's *T*-test). Thus, the block of N-type Ca^{2+} channels decreased the release probability at corticostriatal terminals. Representative records at right. Numbers refer to recording time at left. Horizontal bar indicates time of ligand superfusion. (B) The paired pulse ratio was enhanced in the presence of 400 nM ω-agatoxin TK, from 1.04 (0.9–1.2) in control to 2.7 (2.2–4.0) in the presence of the peptide ($n = 5$; $P < 0.05$, Wilcoxon's *T*-test). Thus, the block of P/Q-type Ca^{2+} channels decreased the release probability at corticostriatal terminals. (C) The paired pulse ratio was reduced by 5 μM Bay K 8644 from 1.16 (1.0–1.5) to 0.64 (0.2–1.2) ($n = 7$; $P < 0.02$; Wilcoxon's *T*-test), indicating that facilitation of L-type Ca^{2+} channels enhanced the release probability at corticostriatal terminals.

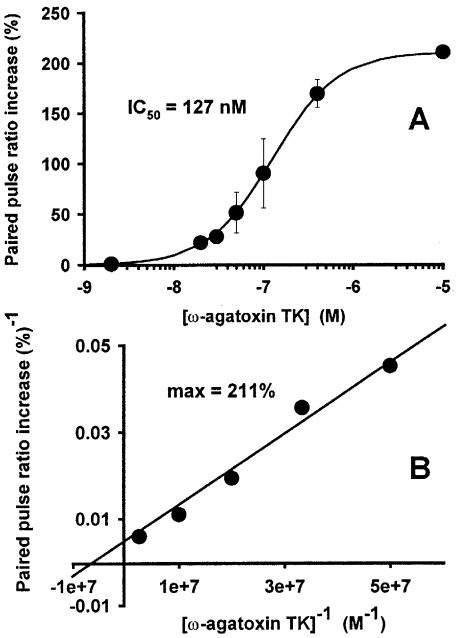

Fig. 3. Concentration–response analysis of ω-agatoxin TK. (A) Concentration–response relationship for ω-agatoxin TK using the increase in percentage of the paired pulse ratio. Plot was built from 24 slices and experiments. Each point is the mean ± S.E.M. of at least four experiments in different slices. (B) A Lineweaver–Burk analysis was used to obtain maximal effect (y-intercept) = 211%, $r = 0.97$. Maximal response obtained with the double reciprocal plot was used to constrain the Marquardt fit of the Hill equation in A to obtain $IC_{50} = 127$ nM and $nH = 1.2$, $r = 0.92$. Maximal response was achieved with concentrations higher than 1 μM ω-agatoxin TK (which were not tested).

with other preparations using different methods (Wheeler et al., 1994; Teramoto et al., 1995, 1997).

3.4. Bay K 8644

Previous work has shown that the block of L-type Ca^{2+} channels does not affect corticostriatal transmission significantly (Hill and Brotchie, 1999; Bargas et al., 1998; Lovinger et al., 1994; Turner et al., 1993). This is also true for different types of central nerve terminals (Dunlap et al., 1995; Wu and Saggau, 1997; Randall and Benham, 1999). We confirmed this point by using the paired pulse ratio: 5 μM nitrendipine did not enhance the paired pulse ratio significantly (not shown; $n = 3$). In spite of this negative result, we decided to test Bay K 8644 (5 μM), a selective agonist of L-type Ca^{2+} channels, to see what happens when L-type Ca^{2+} channels are *facilitated* instead of *blocked*. Fig. 2C shows that Bay K 8644 reduced the paired pulse ratio significantly from a mean ± S.E.M. = 1.25 ± 0.07, median = 1.16, range = 1.0–1.5, in the controls, to 0.65 ± 0.1, median = 0.64, range = 0.2–1.2, in the presence of Bay K 8644 ($n = 7$; $P < 0.02$; Wilcoxon's T-test). This is a 45% reduction (calculated from medians) from the control, similar to that produced by high external Ca^{2+} concentrations (see above and Fig. 1A), suggesting that Bay K 8644 increases Ca^{2+} entry at the terminals.

4. Discussion

The *rationale* of the paired pulse ratio protocol has been previously discussed and validated (e.g., Katz and Miledi, 1968; Dunwiddie and Hass, 1985; Zucker, 1989, 1999; Gerdeman and Lovinger, 2001). The types of Ca^{2+} channels involved in glutamate release at the corticostriatal terminals have been previously studied with different methods (Hill and Brotchie, 1999; Bargas et al., 1998; Lovinger et al., 1994; Turner et al., 1993). As in other systems (Wu and Saggau, 1997; Randall and Benham, 1999), N- and P/Q-type channels, but not L-type Ca^{2+} channels, are in charge of triggering transmitter release. Based on this previous knowledge, in this work, the paired pulse ratio protocol was used for concentration–response analysis of ω-agatoxin TK on corticostriatal terminals, to determine whether the blocking profile obtained is more similar to that for the P- or the Q-type channel.

This analysis was compared to a concentration–response analysis of Cd^{2+} action. In addition, a comparison between ω-agatoxin TK and ω-conotoxin GVIA, using the paired pulse ratio, showed that the most potent peptide was ω-agatoxin TK. It is concluded that, under the present experimental conditions, Q-type channels appear to be the most important channel for transmitter release at these terminals.

4.1. The paired pulse ratio in the corticostriatal preparation

The paired pulse ratio increased when the release probability was low and decreased when the release probability was high. Since the neostriatum is not a laminar structure like the hippocampus or cortex, these experiments (Fig. 1A,B) show that the protocol is still valid for non-laminar structures provided that excitatory transmission is isolated (with bicuculline). These experiments support the results discussed below.

Cd^{2+} acted in the micromolar range ($IC_{50} = 13$ μM), with a Hill coefficient that suggested that the ion does not distinguish between different high-voltage activated Ca^{2+} channels. Saturation was achieved with concentrations higher than 50 μM. These values are similar to those found with different techniques in other preparations (reviewed in Wu and Saggau, 1997).

4.2. Peptidic Ca^{2+} channel antagonists

Both ω-conotoxin GVIA and ω-agatoxin TK enhanced the paired pulse ratio. Therefore, Ca^{2+} entry through N- and P/Q-types of Ca^{2+} channel regulates neurotransmitter release at corticostriatal terminals (Hill and Brotchie, 1999; Bargas et al., 1998; Lovinger et al., 1994; Turner et al., 1993).

The results also indicated that ω-agatoxin is a more potent blocker of transmission than ω-conotoxin GVIA, at corticostriatal terminals. This is the case for other mammalian central nerve terminals involved in fast transmission (Takahashi and Momiyama, 1993; Wheeler et al., 1994; Teramoto et al., 1995, 1997; Iwasaki and Takahashi, 1998; Iwasaki et al., 2000). Although N- and P/Q-type Ca^{2+} channels regulate transmitter release in different classes of embryonic central neurons (Reid et al., 1998; Iwasaki et al., 2000), some neurons suffer a developmental switch, so that only P/Q-type channels remain in the mature cells (Iwasaki and Takahashi, 1998; Iwasaki et al., 2000). In contrast, some other neurons keep using both N- and P/Q-type Ca^{2+} channels after reaching maturity. Among the later are the pyramidal cortical and hippocampal neurons (Dunlap et al., 1995; Wu and Saggau, 1997; Iwasaki et al., 2000). The corticostriatal terminals come from pyramidal neurons. Results have shown that the paired pulse ratio protocol is able to detect the involvement of both N- and P/Q-type channels in corticostriatal transmission and, moreover, the relative importance of each channel type (Takahashi and Momiyama, 1993; Tarelius and Breer, 1995; Wu and Saggau, 1997; Randall and Benham, 1999; Iwasaki et al., 2000; Dooley et al., 2000). Furthermore, the concentration–response analysis of ω-agatoxin TK showed a Q-type, not a P-type, profile (IC_{50} = 127 nM). In addition, the Hill coefficient for ω-agatoxin was not significantly different from 1.0, suggesting that one molecular target is affected. This agrees with previous electrophysiological findings in pyramidal cells (Takahashi and Momiyama, 1993; Wheeler et al., 1994). Moreover, lower concentrations of agatoxin, included in the concentration–response analysis, did not change the Hill coefficient, which according to Dunlap et al. (1995) is expected to be significantly larger than 1.0 if cooperativity from hidden (e.g., P-type) channels is present. However, experiments to completely rule out the participation of P-type Ca^{2+} channels under all circumstances were out of the scope of this report. On the basis of the effects of an L-type channel agonist (see below), it is fair to say only that Q-type channels appear to be the most important when orthodromic responses are evoked with moderate stimulus strength (see Methods). Thus, for example, a recent report states that both P- and Q-type Ca^{2+} channels participate in the 4-aminopyridine-evoked release of glutamate from striatal synaptosomes (Hill and Brotchie, 1999). 4-Aminopyridine increases the release efficacy at these and other terminals (Flores-Hernandez et al., 1994). Thus, it may be that P-type channels play a role during strong stimulation or trains of stimuli, where release reliability is at stake. This might be important given the two modes of action of these neurons (Wilson, 1993). Alternatively, thalamic terminals may use P-type Ca^{2+} channels for release (Iwasaki et al., 2000), and synaptosomal preparations are from both thalamus and cortex. Further experiments are needed to elucidate these points.

4.3. A role for L-type Ca^{2+} channels?

A decrease in the paired pulse ratio was produced by the L-type Ca^{2+} channel agonist, Bay K 8644 (Fig. 2C), similar to that produced by high external Ca^{2+} concentrations (Fig. 1A,B), suggesting that this agonist increases Ca^{2+} entry into the terminals. This was surprising because block of L-type Ca^{2+} channels does not affect release significantly (Hill and Brotchie, 1999; Bargas et al., 1998; Lovinger et al., 1994; Turner et al., 1993). Nevertheless, Ca^{2+} currents of the L-type can be disclosed at synaptic terminals (Urbano and Uchitel, 1999), even when their blockage does not affect release. Moreover, dihydropyridines enhance evoked release under certain conditions (Rosato-Siri and Uchitel, 1999). It is, therefore, the blocking action on release that is absent. This asymmetry of the action of dihydropyridines suggests that although L-type Ca^{2+} channels do not participate in the release process, they still could participate in its modulation through some transmitter that enhances L-type Ca^{2+} currents. This point deserves further investigation.

Acknowledgements

Authors are grateful to M.C. Vilchis for her technical assistance. J. Barral was a fellow of PASPA-DGAPA-UNAM and E. Mendoza was a fellow of PROBETEL-UNAM. This work was supported by the following grants to J. Bargas: DGAPA-UNAM: IN202300; CONACyT: 31839-N; FIRCA-NIH Grant: RO3 PWO1214-01 and The Millenium Scientific Initiative: W-8072.

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