Two types of low-voltage-activated Ca^{2+} channels in neurones of rat laterodorsal thalamic nucleus

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1. The pharmacological and kinetic properties of two types of low-voltage-activated (LVA) Ca^{2+} currents were studied in thalamocortical neurones of the laterodorsal (LD) thalamic nucleus during early postnatal development. The whole-cell patch-clamp technique was used on brain slices from rats of three age groups: 12, 14 and 17 days old (postnatal day (P) 12, P14 and P17).

2. In P12 neurones, the population of LVA Ca^{2+} channels was homogeneous. LVA Ca^{2+} current elicited by depolarizing voltage steps from a holding potential more negative than −70 mV was sensitive to nifedipine ($K_d = 2{\cdot}6$ μM). This current reached a maximum at about −55 mV and had a fast monoexponential decay with a time constant, $\tau_{h,f}$, of 32·3 ± 4·0 ms.

3. The population of LVA Ca^{2+} channels in P14 and P17 neurones was found to be heterogeneous. A subpopulation of nifedipine-insensitive LVA Ca^{2+} channels was observed. The current–voltage curve of the Ca^{2+} current had a characteristic hump with two peaks at about −65 and −55 mV. As well as the fast component (designated $I_{T,f}$), the decay of the LVA current also included a slow component (designated $I_{T,s}$), with inactivation time constants ($\tau_{h,s}$) of 54·2 ± 4·5 and 68·6 ± 3·17 ms for P14 and P17 neurones, respectively.

4. The kinetics of both components could be well approximated by the m^2h Hodgkin–Huxley equation. No significant difference in activation kinetics was observed. The activation time constants for the fast ($\tau_{m,f}$) and slow ($\tau_{m,s}$) components were 6·3 ± 1·0 and 7·3 ± 1·5 ms, respectively.

5. La^{3+} at a concentration of 1 μM effectively blocked the $I_{T,f}$ component but Ni^{2+} (25 μM) completely eliminated the $I_{T,s}$ component.

6. Steady-state inactivation curves of both components could be best fitted by a Boltzmann function with membrane potential values at half-maximal inactivation of −85·5 and −98·1 mV for the fast and slow components, respectively.

7. It was concluded that two different subtypes of LVA Ca^{2+} channel are present in LD neurones. Only the fast type is well expressed at the earliest postnatal stage (P12). The slow type could be found at the end of the second week (P14). The amplitude of the slow current increased progressively up to P17, obviously coinciding with dendritic expansion as judged by progressive increase of the membrane capacitance of the corresponding neurones. This property appears to differentiate neurones of the associative nuclei from neurones of other thalamic nuclei.

The functional properties of thalamic neurones play a major role in the organization of rhythmic thalamocortical activity. Thalamic associative nuclei, including the laterodorsal (LD) nucleus, are of special importance in this respect; however, it is not yet clear what specific features of neuronal processes are most important for the functions of the neurones. It has been shown previously that functional specificity of the neurones in other thalamic nuclei (nucleus reticularis, ventrobasal complex) is mainly due to the expression of low-voltage-activated (LVA) Ca^{2+} channels in these neurones (Coulter, Huguenard & Prince, 1989; Huguenard & Prince, 1992; Tsakiridou, Bertollini, De Curtis, Avanzini & Pape, 1995). Although discovered quite a long time ago (Veselovsky & Fedulova, 1983), the LVA Ca^{2+} channels still form a somewhat mysterious group of channels: in many excitable cells they can be detected only during a short

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period of their life cycle; the conditions for their physiological activation are not quite clear; and no corresponding α_1-subunit has yet been identified. Previous investigations of neurones freshly isolated from the ventromedial hypothalamic complex of rats have indicated that LVA Ca^{2+} channels in this complex are substantially different from T-type Ca^{2+} channels in peripheral neurones, especially in their pharmacological properties (Akaike, Kostyuk & Osipchuk, 1989).

Therefore, we have performed a special study of the functional and pharmacological properties of LVA Ca^{2+} channels in neurones of the LD thalamic nucleus *in situ* using the whole-cell path-clamp technique. Preliminary results of the study have appeared in abstract form (Kostyuk, Tarasenko & Eremin, 1995).

METHODS

Preparation of brain slices

Experiments were carried out on three age groups of Wistar rats: 12, 14 and 17 days old (postnatal day (P) 12, P14 and P17; the day of birth was designated as P1). The animals were decapitated and the brain was removed and placed in cold (4 °C) physiological saline for 3–4 min. It was then separated into two hemispheres, and one of them was cut in the sagittal plane using a vibrating slicer. Slices (300 μm thick) were immediately transferred into physiological saline containing (mM): NaCl, 125; KCl, 2·2; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 24; NaH_2PO_4, 1·25; and glucose, 15 (pH 7·4); bubbled with 95% O_2–5% CO_2. After incubation for 1 h at 32 °C and 1 h at room temperature (about 22 °C), the chosen slice was transferred into the recording chamber. An upright microscope (Ergoval; Carl Zeiss) with a ×3·2 Semiplan objective lens was used to observe the upper surface of the slice and identify the thalamic nuclei; a ×20 Leitz Wetzlar objective lens was used to identify the cells. The LD thalamic nucleus was identified according to the rat brain stereotaxic atlas (Paxinos & Watson, 1944).

Solutions and drugs

For the isolation of Ca^{2+} currents, the external solution contained (mM): choline chloride, 113; TEA-Cl, 27; $CaCl_2$, 2; $MgCl_2$, 0·5; Tris-Cl, 20; pH adjusted to 7·4 with Tris-OH. Tetrodotoxin was added to this solution at a final concentration of 1 μM, a concentration which completely blocked voltage-operated Na^+ channels. To suppress K^+ currents, the pipette solution contained (mM): CsCl, 130; $MgCl_2$, 5; TEA-Cl, 10; $CaCl_2$, 1; EGTA, 10; and Tris-Cl, 10; pH adjusted to 7·3 with CsOH. To study the pharmacological properties of Ca^{2+} channels, the following substances were used: nifedipine (Sigma) dissolved in dimethyl sulphoxide, $LaCl_3$ and $NiCl_2$. These drugs were added to the external solution at the concentrations indicated in the Results. All external solutions tested were applied via a pressure-ejection micropipette (tip diameter, 40–50 μm), which was located near the patched neurone.

Electrophysiological recordings and data analysis

Thalamocortical LD neurones were voltage clamped at room temperature using the whole-cell configuration of the patch-clamp technique (Hamill, Marty, Neher, Sackmann & Sigworth, 1981). Since membrane surface area increases during postnatal development, the inner diameters of pipette tips made from soft glass varied from 2 to 3·5 μm depending on the size of the cell studied. When filled with the pipette solution, the pipette resistance was 2–5 MΩ. Current recordings were obtained with an analog of the List EPC/5 patch-clamp amplifier.

The passive electrical properties of the neurones tested were measured by analysing the capacitive transients and leakage currents during seal formation and after breaking the cell membrane. The pipette electrode capacitance (C_p) and whole-cell capacitance were estimated from the integral of the corresponding transient capacitive currents evoked by a 10 mV hyperpolarizing step from a holding potential (V_h) of −80 mV. Only cells in which the decay of whole-cell capacitive transient could be fitted by one exponential were used in the final analysis. In this case, the cell could be treated as a single isopotential compartment (Kay & Wong, 1987). The membrane capacitance (C_m) was calculated by subtracting C_p from the whole-cell capacitance.

Values of series resistance (R_s) were determined in two different ways using the time constant (τ) of the capacitive transient recording after breaking the membrane ($R_s = \tau/C_m$) and dividing the amplitude of capacitive currents by the change in voltage. Cells were discarded if the voltage drop evaluated as a product of R_s and the amplitude of the LVA Ca^{2+} current was more than 6 mV.

Voltage-clamp control was considered to be satisfactory if: (1) voltage-dependent current activation was smooth; (2) no excessive delay in the onset of current was observed; and (3) the onset and offset kinetics depended on voltage but not on the amplitude of the current. Only cells in which adequate clamp conditions were obtained using these criteria were included in the kinetic study.

Compensation of leakage currents was achieved by using an additional electrical circuit during the recording session. Depolarization steps were applied if the current trace elicited at the onset of a 10 mV hyperpolarizing command potential from the holding potential coincided with the zero line.

Ca^{2+} currents were elicited by a series of increasing depolarizing steps every 5–7 s. The appropriate V_h was specified for each experiment. Ca^{2+} currents were filtered at 1 kHz (−3 dB, eight-pole active Bessel filter), digitally sampled at 8 μs intervals, stored and analysed with an IBM-PC computer. The recordings were delayed for at least 5 min after breaking the cell membrane to allow the membrane currents to stabilize before collecting data. The current amplitude was measured as the difference between peak inward current and zero current. Other specific protocols are described in the text or in the figure legends. Graphing and curve fitting were performed by SigmaPlot 5.0 (Jandel Scientific GmbH, Erkath, Germany). Time constants and current kinetics were described by fitting with a Hodgkin–Huxley model using methods identical to those described elsewhere (Coulter *et al.* 1989). Results are given as means ± S.E.M.

RESULTS

Passive properties of thalamocortical LD neurones

The passive properties of thalamocortical LD neurones were obtained from analysis of the capacitive transients and leakage currents evoked in response to hyperpolarizing command pulses of 10 mV from a V_h of −80 mV. No age-related difference in leakage currents was seen. As a rule, the leakage current was 20–40 pA. The input resistance of the cells tested was 250–500 MΩ. This resistance was less

than the input resistance of isolated pyramidal neurones of the CA1 region (more than 500 MΩ) of the mature guinea-pig hippocampus (Kay & Wong, 1987) and dopaminergic neurones (779 ± 218 MΩ) of rat substantia nigra compacta in slice preparations (Kang & Kitai, 1993). As described in Methods, the decay of the capacitive transients of cells studied (measured after breaking the cell membrane) could be fitted by a single exponential curve; the time constant of decay varied for different age groups from 0·8 to 2·2 ms. Typical capacitive transients of the neurones of the different age groups tested are shown in Fig. 1*A*. The series resistance determined from the capacitive transients did not exceed 8–14 MΩ for P12 neurones and was significantly smaller (4–8 MΩ) for P17 neurones. Figure 1*B* demonstrates the dependence of the membrane capacitance on the stage of postnatal development. The membrane capacitance progressively increased from 84 ± 3·4 pF ($n = 8$) in P12 neurones to 107 ± 11·3 ($n = 10$) and 156 ± 15·6 pF ($n = 10$) in P14 and P17 neurones, respectively. Since the somatic capacitance in different isolated cells, including thalamocortical neurones, is not more than 15–20 pF (Murchison & Griffith, 1995; Tsakiridou *et al.* 1995), such an increase in membrane capacitance appears to be connected with the growth of the dendritic tree and corresponding expansion of the cell surface.

General properties of Ca^{2+} currents

Typical records of Ca^{2+} currents obtained from the neurones of each age group are shown in Fig. 2. Depolarization of the membrane between −80 and −45 mV from a V_h of −95 mV evoked a transient Ca^{2+} current, while after a depolarization of up to −45 mV a sustained Ca^{2+} current was found, which reached a maximum at about −15 mV, confirming the existence of LVA and high-voltage-activated (HVA) Ca^{2+} currents in LD neurones. However, a comparison of the basic characteristics of LVA Ca^{2+} currents in the neurones of the different age groups demonstrated a striking age-related difference. In P12 neurones, the population of LVA Ca^{2+} channels appeared to be homogeneous; the current–voltage (I–V) relationship of the Ca^{2+} current had one peak at about −55 mV and a second peak at about −15 mV (Fig. 2*A*). On the other hand, the I–V relationships of Ca^{2+} currents in P14 and P17 neurones had peaks at about −65 and −55 mV (Fig. 2*B* and *C*). The presence of two peaks in the I–V curves observed in the region of potentials more negative than the maximum HVA Ca^{2+} current may be explained either by the existence of two different types of LVA Ca^{2+} channel or by a negative shift of the I–V curve of dendritic LVA current associated with an error in voltage control in more mature neurones. To clarify this question, a special pharmacological and kinetic study of LVA Ca^{2+} current in neurones of rats of different age groups was performed.

Pharmacological separation of LVA current components

An important feature of LVA Ca^{2+} channels in the P12 neurones tested was their high sensitivity to dihydropyridines (nifedipine), in contrast to LVA T-type channels in many other excitable cells (Griffith, Taylor & Davis, 1994; Fisher & Bourque, 1995). It has been shown previously that nifedipine is an effective blocker of LVA Ca^{2+} channels in hypothalamic neurones (P7) isolated from rats (Akaike *et al.*

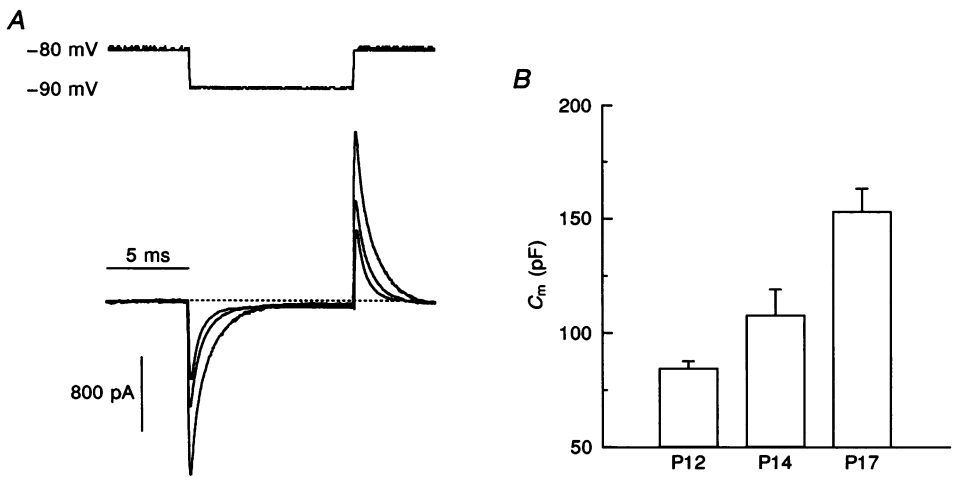

Figure 1. Passive properties of LD neurones

A, transient capacitive currents evoked at the onset of a 10 mV hyperpolarizing command potential from a V_h of −80 mV in P12, P14 and P17 neurones. The voltage protocol is shown at the top. Constant leakage current for the data presented was about 30, 25 and 27 pA for P12, P14 and P17 neurones, respectively. The decay of the capacitive current was fitted by a single exponential curve with time constants of 1·16, 1·3 and 1·15 ms for P12, P14 and P17 neurones, respectively. Series resistance was 14, 12 and 8 MΩ and membrane capacitance (C_m) was 83, 106 and 142 pF for P12, P14 and P17 neurones, respectively. *B*, change in membrane capacitance as a function of the postnatal developmental stage of the neurones tested. Membrane capacitance values are presented as means + S.E.M.

1989). In our experiments, nifedipine at a concentration of 0·1 μM did not significantly reduce LVA Ca^{2+} currents in neurones of all age groups (about 5% reduction; $n = 14$). As the concentration of nifedipine was increased above 1 μM the LVA Ca^{2+} current progressively decreased. Application of 100 μM nifedipine caused reversible block (93 ± 5% reduction; $n = 7$) of the LVA Ca^{2+} current in P12 cells. In contrast, the LVA Ca^{2+} current could not be completely suppressed in P14 cells even by the application of 100 μM nifedipine for 20 min. More than 10% ($n = 7$) of the control current was observed (Fig. 3*A*). A dose–response curve of the inhibitory effect of nifedipine on the LVA Ca^{2+} current of P14 cells is shown in Fig. 3*B*. In P17 cells, the nifedipine-insensitive component (current in the presence of 100 μM of the drug) increased in comparison with the P14 cells and was 25 ± 4% of the control current ($n = 6$). The nifedipine-sensitive component could be obtained by subtraction of the LVA current obtained after blocking by nifedipine from the total current (Fig. 3*C*). The decay time constant of the nifedipine-sensitive component was definitely less than that of the nifedipine-insensitive component (Fig. 3*D*).

The finding of such a prominent difference in the pharmacological sensitivity of two components of the LVA Ca^{2+} current prompted us to compare their sensitivity to other LVA Ca^{2+} channel blockers, especially di- and trivalent cations. We found that La^{3+} blocked the LVA Ca^{2+} current at a concentration of 0·1 μM (57 ± 5% reduction for P12 neurones; $n = 13$). It appeared to be a potent specific blocker of the nifedipine-sensitive component, leaving the nifedipine-insensitive component unaffected. An example of the blocking effect of La^{3+} (1 μM; P17 neurone) is shown in Fig. 4*A*. The La^{3+}-sensitive component was obtained by subtraction of the current obtained after application of La^{3+} from the total current (Fig. 4*B*). In contrast, Ni^{2+} (a specific blocker of the well-known T-type channels in other tissues) at a concentration of 25 μM decreased the nifedipine-insensitive component (Fig. 4*C*), leaving only the nifedipine-

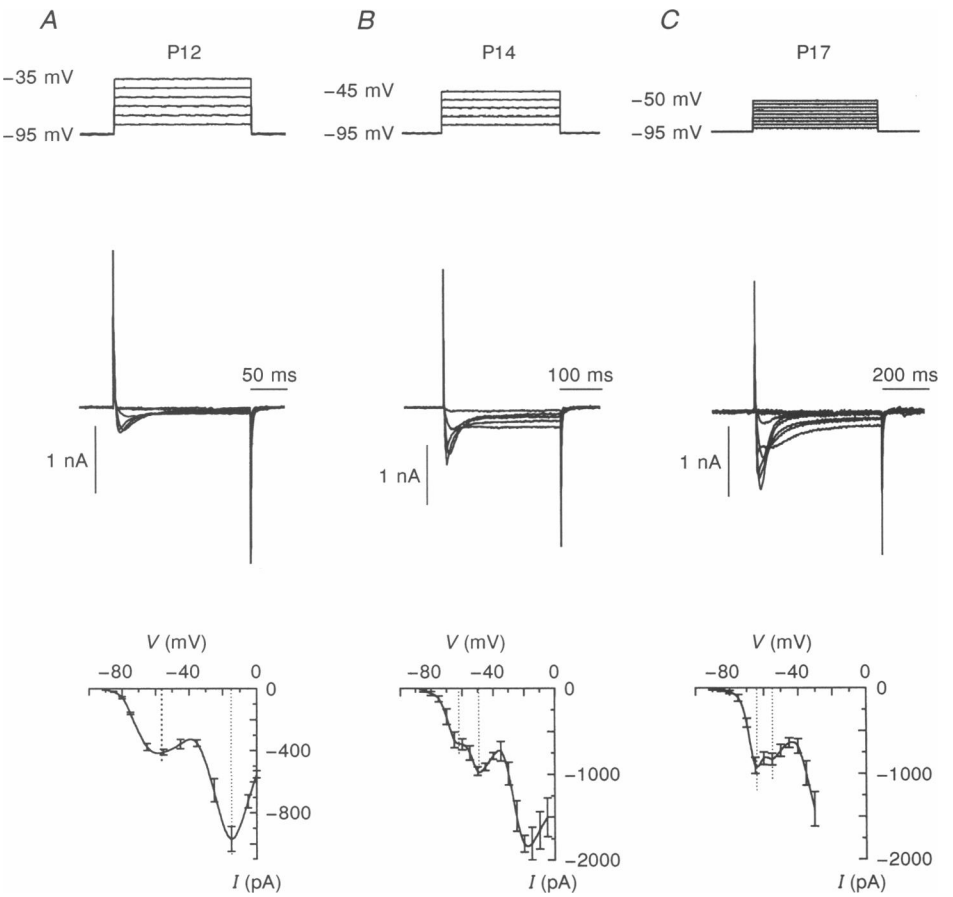

Figure 2. Whole-cell Ca^{2+} current in P12, P14 and P17 neurones

A–C, depolarizing voltage steps from −95 mV for P12 (*A*), P14 (*B*) and P17 (*C*) neurones evoked a transient LVA Ca^{2+} current followed by a sustained HVA Ca^{2+} current at more positive potentials with a maximum at about −15 mV. In each panel the voltage protocols are shown at the top. *I–V* relationships of peak currents for P12, P14 and P17 neurones are shown beneath the current traces. Note the typical hump in the *I–V* curve for P12 neurones, separating the maximum LVA and HVA Ca^{2+} currents. *I–V* relationships of Ca^{2+} current for P14 and P17 neurones, as well as a peak of HVA Ca^{2+} current at about −15 mV, had two peaks with the maximum at about −65 and −55 mV. Mean data from 7 cells for each age group were used to construct the *I–V* curves.

sensitive component (Fig. 4*D*). Nifedipine (100 μM) completely eliminated the rest of the LVA Ca^{2+} current ($n = 7$) remaining after the block by Ni^{2+} (25 μM). Thus, pharmacological studies definitely confirm the suggestion about the presence of different LVA current components in the neurones studied.

Kinetic separation of two components of the LVA Ca^{2+} current

Besides the different pharmacological sensitivity, the LVA current components also demonstrated different kinetic properties. In P12 neurones, only a transient current appeared at membrane depolarization between −75 and −55 mV ($V_h = -95$ mV), reaching a maximum at approximately −55 mV. The LVA Ca^{2+} current induced by membrane depolarization from −95 to −60 mV had a monoexponential decay and declined fast and completely in about 150 ms (Fig. 5*A*). An analysis of the activation and inactivation time courses of the current indicated that it could be well fitted with the m^2h Hodgkin–Huxley equation, as has already been shown for the activation kinetics of other types of Ca^{2+} currents (Kostyuk, Krishtal,

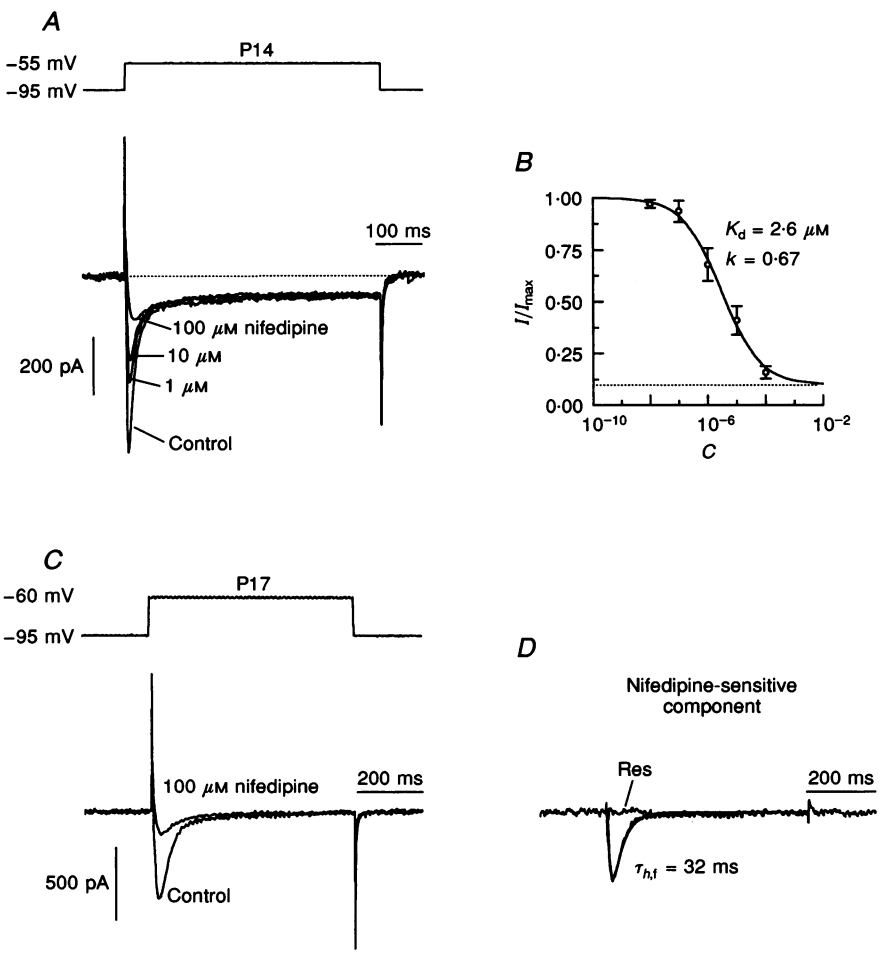

Figure 3. Action of nifedipine on LVA Ca^{2+} current in P14 and P17 LD neurones

A, decrease in amplitude of the LVA Ca^{2+} current with increasing concentrations of nifedipine. LVA Ca^{2+} currents for P14 neurones before (Control) and after blocking by 1, 10 and 100 μM nifedipine are shown. V_h was −95 mV with a 40 mV step. LVA Ca^{2+} currents were elicited by step depolarization every 7 s. *B*, the dose–response relationship of LVA Ca^{2+} currents of P14 neurones was fitted by a sigmoidal curve (the modified Boltzmann function used was: $I/I_{max} = 1/(1 + a + \exp((\log C - \log K_d)/k)) + b$, where C is the drug concentration, K_d is the half-inactivation concentration, I_{max} is the amplitude of the control current, I is the current amplitude after blocking by nifedipine, b is a sustained ratio, a is a normal coefficient and k is the slope factor) with $K_d = 2{\cdot}6$ μM, and $k = 0{\cdot}67$. The LVA Ca^{2+} currents obtained after addition of nifedipine were normalized to the control Ca^{2+} current. All points show the mean data from 7 neurones. *C*, LVA Ca^{2+} current for P17 neurones after 20 min application of 100 μM nifedipine (nifedipine-insensitive current). V_h was −95 mV with a 35 mV step. The modified Hodgkin–Huxley equation fitting the nifedipine-insensitive current (designated $I_{T,s}$) had time constants of activation ($\tau_{m,s}$) and inactivation ($\tau_{h,s}$) of 7 and 67 ms, respectively. *D*, the nifedipine-sensitive component was obtained by subtracting LVA Ca^{2+} current after block by 100 μM nifedipine from the total LVA Ca^{2+} current. It was fitted in terms of the m^2h model with the inactivation time constant ($\tau_{h,f}$) indicated.

Pidoplichko & Shakhovalov, 1979; Kay & Wong, 1987). The equation used for fitting the LVA Ca^{2+} current (I_{Ca}) was:

$$I_{Ca} = A(1 - \exp(-t/\tau_m))^2(\exp(-t/\tau_h) - I_\infty), \quad (1)$$

where t is time, τ_m and τ_h are the activation and inactivation time constants, respectively, I_∞ is a sustained Ca^{2+} current, and A is an amplitude scaling factor. As we showed above, the current was sensitive to nifedipine and was best fitted in terms of the m^2h model with parameters $\tau_{m,f}$ and $\tau_{h,f}$ of 6·3 ± 1·0 and 32·3 ± 4·0 ms, respectively ($n = 15$). This nifedipine-sensitive current was designated $I_{T,f}$ ('fast') component.

In both P14 and P17 neurones, however, the decline of the LVA current was more complicated and included a slow component lasting hundreds of milliseconds (Fig. 5*B*). As shown above, this component could be obtained in a pure form after blocking by nifedipine. The decline of the nifedipine-insensitive component was a monoexponential with time constants of 54·2 ± 4·5 ($n = 26$) and 68·6 ± 3·17 ms ($n = 18$) for P14 and P17 cells, respectively; therefore, this component was designated $I_{T,s}$ ('slow') and could also be approximated by the m^2h model. No substantial difference in the activation time constants of the $I_{T,f}$ and $I_{T,s}$ components was found. The value of the activation time constant for the slow component was 7·3 ± 1·5 ms ($n = 44$) (mean value for both P14 and P17 cells).

Steady-state inactivation of the LVA current components

A summary of the analysis of the inactivation characteristics of the $I_{T,f}$ component is shown in Fig. 6*A*. The LVA Ca^{2+} currents were obtained by membrane depolarization to −55 mV from different V_h values between −95 and −75 mV. The steady-state inactivation curve (Fig. 6*B*) could be best fitted by a Boltzmann function with a membrane potential

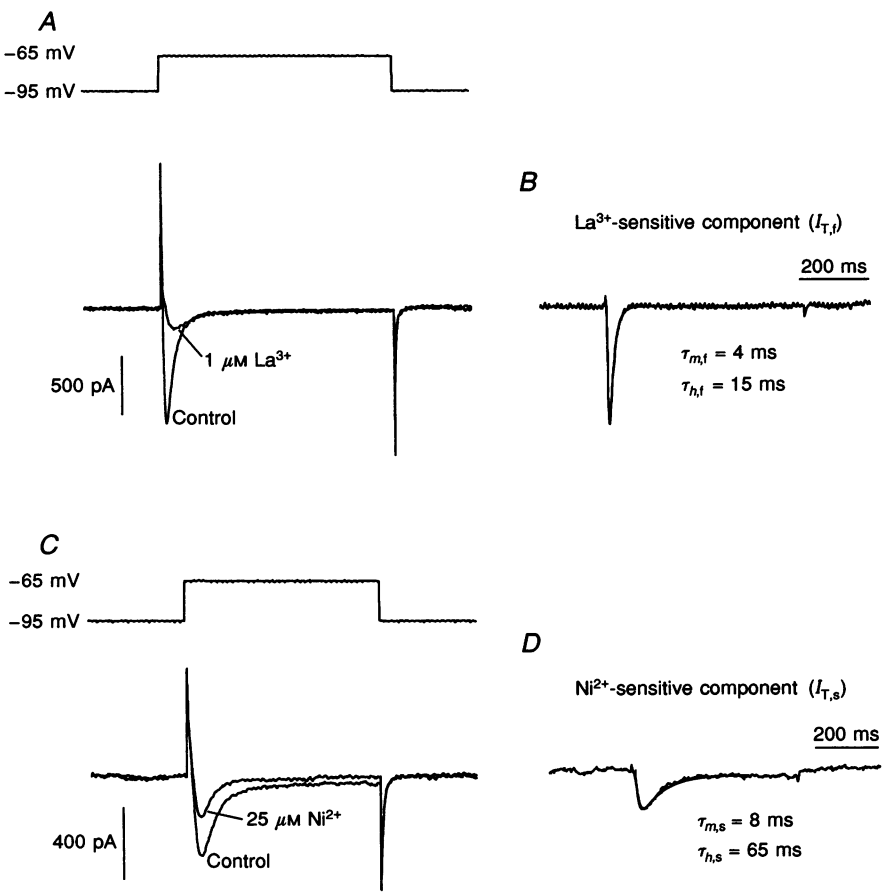

Figure 4. Pharmacological properties of different components of the LVA Ca^{2+} current

A, LVA Ca^{2+} current (P17 neurone) recorded before (Control) and in the presence of 1 μM La^{3+}. The resistant LVA Ca^{2+} current could be best fitted by the m^2h equation with $\tau_{m,s}$ of 8 ms and $\tau_{h,s}$ of 69 ms. *B*, La^{3+}-sensitive component obtained by subtraction of the LVA Ca^{2+} current recorded after the addition of La^{3+} from the control current. The values of the activation ($\tau_{m,f}$) and inactivation ($\tau_{h,f}$) time constants are indicated. *C*, LVA Ca^{2+} current before (Control) and after application of 25 μM Ni^{2+} (P14 neurone). *D*, the Ni^{2+}-sensitive component ($I_{T,s}$) was obtained as the difference between the control current and current obtained after application of Ni^{2+}. A fitted curve is superimposed on the $I_{T,s}$ component with the parameters indicated. In *A* and *C* the voltage protocol is shown at the top; $V_h = -95$ mV.

value at half-maximal inactivation ($V_{½}$) of −85·5 mV and a slope factor (k) of 2·8 mV ($n = 6$). Thus, the $I_{T,f}$ component showed almost complete inactivation at a V_h of −75 mV.

The threshold depolarization necessary to activate the $I_{T,s}$ component was less than that required to activate the $I_{T,f}$ component (about −80 mV; Fig. 6*C*). The steady-state inactivation curve of this component was definitely shifted in the hyperpolarizing direction compared with the curve for $I_{T,f}$ (Fig. 6*D*). The parameters of this curve were: $V_{½} = -98·1$ mV and $k = 5·28$ mV ($n = 7$). However, it was difficult to estimate precisely the depolarization level yielding maximal current amplitude, since the HVA Ca^{2+} current became superimposed on the LVA current at depolarizations exceeding −50 mV. The HVA current was present in all neurones tested, and its amplitude varied substantially between different cells; peak amplitude was reached at about −15 mV.

Changes in expression of different LVA current components with neuronal development

On the basis of the pharmacological separation of LVA current we could estimate the amplitude of each component in the different age groups. From a summary of the data obtained it could be concluded that dramatic changes in the expression of different LVA Ca^{2+} components occurred during early neuronal development. Only the $I_{T,f}$ component was expressed during the early postnatal period (Fig. 7). The amplitude of $I_{T,f}$ increased from 389 ± 47 pA ($n = 15$) for P12 neurones to 670 ± 74 pA ($n = 18$) for P17 neurones. In contrast, the $I_{T,s}$ component appeared only at the end of the second week. The amplitude of this component increased progressively from 100 ± 9 pA for P14 ($n = 26$) up to 630 ± 58 pA for P17 neurones ($n = 18$).

No age-related difference in the current density of $I_{T,f}$ was seen. The current density was 4·6 ± 0·5 pA pF^{-1} for P12 ($n = 15$), 4·9 ± 0·6 pA pF^{-1} for P14 ($n = 26$) and 4·3 ± 0·4 pA pF^{-1} for P17 cells ($n = 18$). In contrast, the current density of $I_{T,s}$ increased significantly from 0·9 ± 0·1 pA pF^{-1} for P14 to 4·1 ± 0·4 pA pF^{-1} for P17 cells. This new type of Ca^{2+} channel began to be expressed at P12–14. The appearance of Ca^{2+} channels with different pharmacological and kinetic properties appears to give the neurones of associative nuclei a specific quality that differentiates them from neurones of other thalamic nuclei.

DISCUSSION

The present study demonstrates that LVA Ca^{2+} channels are well expressed in LD thalamic neurones. The mean maximal amplitude of the corresponding currents is substantially higher compared with values obtained previously in acutely dissociated neurones from the reticular thalamic nucleus (Huguenard & Prince, 1992; Tsakiridou *et al.* 1995) or the ventrobasal thalamic complex (Coulter *et al.* 1989) as well as from the ventromedial hypothalamic region (Akaike *et al.* 1989). It should be taken into account that results obtained from enzymatically treated isolated neurones and neurones in slices may be different; the neurones *in situ* are probably

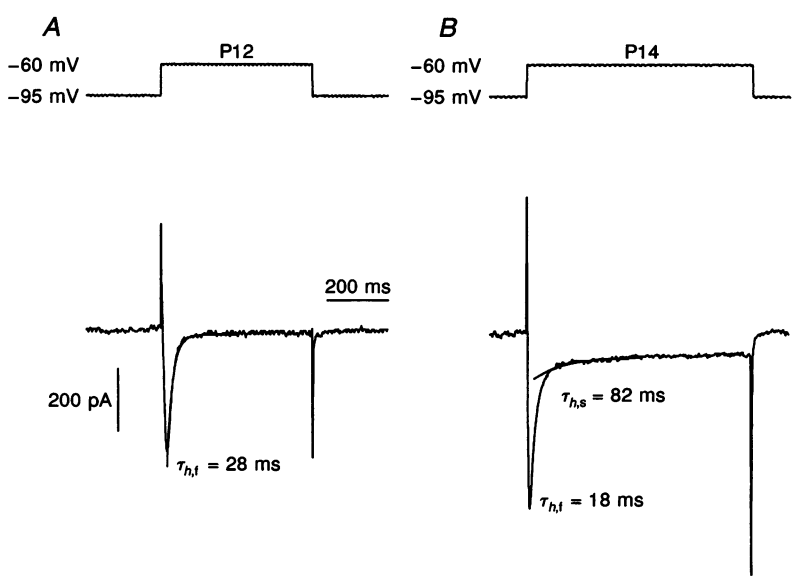

Figure 5. Analysis of the kinetic inactivation of LVA Ca^{2+} current in P12 and P14 LD neurones

A, a transient LVA Ca^{2+} current in a P12 neurone evoked by step depolarization from a V_h of −95 mV to a test potential of −60 mV. The inactivation kinetics could be fitted by a single exponential with $\tau_{h,f}$ of 28 ms. *B*, example of LVA Ca^{2+} current in a P14 neurone, the inactivation being fitted by a double-exponential curve with time constants $\tau_{h,f}$ and $\tau_{h,s}$ of 18 and 82 ms, respectively. In *A* and *B*, the voltage protocol is shown at the top. The duration of the 35 mV step was 600 and 800 ms for P12 and P14 neurones, respectively. Calibration bars in *A* also apply to *B*.

less damaged and retain channels on their dendrites. For example, quite large LVA Ca^{2+} currents were recorded in slices from dopaminergic neurones of substantia nigra (489 ± 170 pA). A special analysis of this problem has been made recently on reticular thalamic neurones, using a comparison of data obtained from isolated (dendrite-free) neurones and neurones in slices, with subsequent computational analysis of the effects of currents originating in distal dendrites (Destexhe, Contreras, Steriade, Sejnowski & Huguenard, 1996), with the conclusion that LVA Ca^{2+} channels are in fact present on distal dendrites of reticular neurones.

In most structures, the expression of LVA Ca^{2+} channels is highly dependent on the postnatal developmental stage. However, this dependence was found to be different in different experiments. In rat dorsal root ganglion (DRG) neurones there is a steep peak in the expression of these channels immediately after birth with a gradual decline during postnatal development (Fedulova, Kostyuk & Veselovsky, 1991). In ventrobasal and reticular thalamic neurones the density of these channels increases up to P15–17, reaching a level of about 510 and 270 pA, respectively (Tsakiridou *et al.* 1995). Such a discrepancy is obviously connected to non-homogeneity of LVA Ca^{2+} channels in different neuronal structures, which has already been indicated by Huguenard & Prince (1992): they noticed that in neurones from the thalamic reticular nucleus the LVA Ca^{2+} currents have slower activation kinetics, and the inactivation rate is almost independent of potential. In some

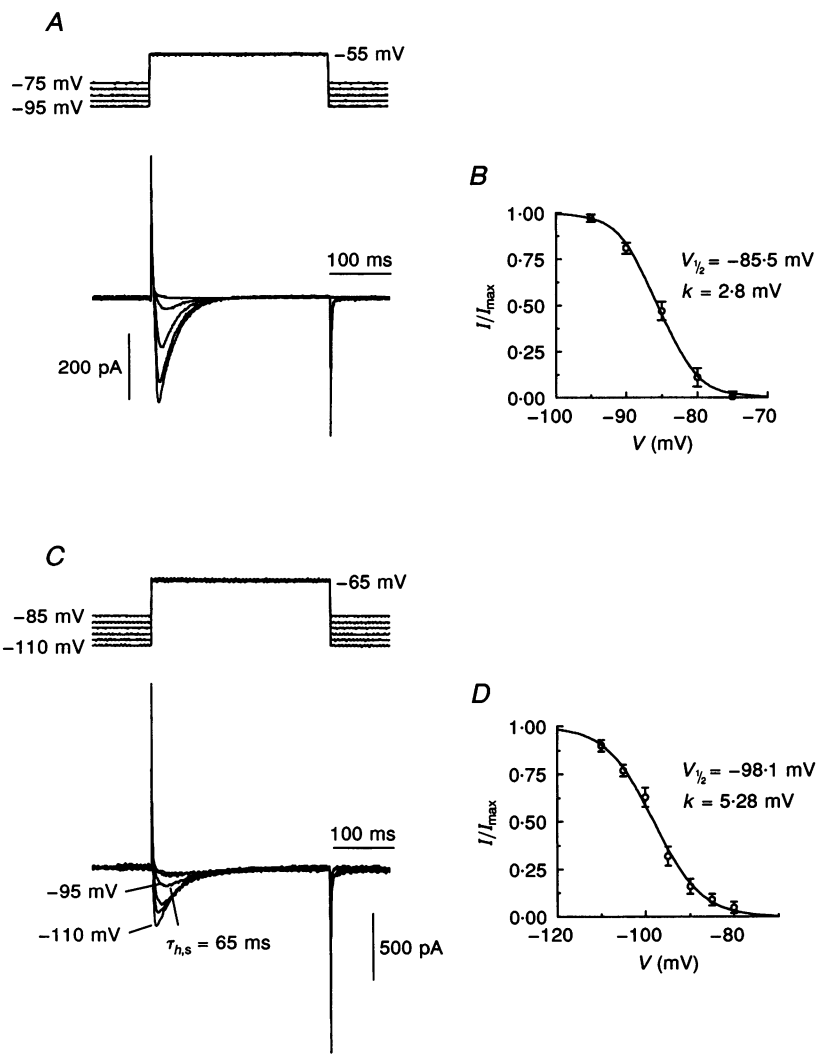

Figure 6. Steady-state inactivation of $I_{T,f}$ and $I_{T,s}$ components

A, voltage dependence of steady-state inactivation of the $I_{T,f}$ component. The membrane was depolarized to −55 mV from different V_h values between −95 and −75 mV. *B*, the inactivation curve could be best fitted by a Boltzmann equation with $V_{½} = -85{\cdot}5$ mV and $k = 2{\cdot}8$ mV ($n = 6$). *C*, pure LVA $I_{T,s}$ component (after application of 100 μM nifedipine), induced by step depolarization to −65 mV from different V_h values between −110 and −85 mV. *D*, steady-state inactivation curve for $I_{T,s}$ component (P17 neurone). Best fit of the curve was obtained with $V_{½} = -98{\cdot}1$ mV and $k = 5{\cdot}28$ mV ($n = 7$).

DRG neurones, LVA Ca^{2+} currents were found to be highly sensitive to dihydropyridines (Formenti, Arrigoni & Mancia, 1993); LVA Ca^{2+} currents with unusually slow inactivation kinetics were also observed (Kobrinsky, Pearson & Dolphin, 1994). In our experiments, we were fortunate to observe both types of LVA Ca^{2+} currents in the same cells, which made the comparison of their properties quite reliable. The data obtained indicate that $I_{T,f}$ and $I_{T,s}$ are generated by two separate sets of Ca^{2+} channels which differ in activation and inactivation kinetics and have completely different pharmacological sensitivity. Both types of channels obviously differ also in their expression during ontogenetic development. Channels producing $I_{T,f}$ are well expressed immediately after birth; later on their density decreases. In this respect they are similar to T-type channels in DRG neurones or muscle fibres; however, they still differ from them in some structural features of the corresponding α_1-subunit, since, in contrast to T-type channels in peripheral structures, they show very high sensitivity to dihydropyridines. The expression of channels associated with $I_{T,s}$ constantly increases during early ontogenesis, and at the end of the second week (which corresponds to the end of neuronal differentiation and formation of neuronal networks) they become dominating in the formation of LVA Ca^{2+} currents. This difference may correspond to the above mentioned specificity in the spatial location of LVA channels. If the channels associated with $I_{T,s}$ are located mainly in the dendrites, an increase in the density of the corresponding current will be expected in parallel with the growth and maturation of the dendritic tree, which occurs during the initial postnatal period. In contrast, maximal density of $I_{T,f}$ during the early postnatal period, when the dendrites are still rudimentary, indicates their predominant somatic location.

As already mentioned, an important question is to what extent the membrane potential in the expanding dendritic tree can be reliably controlled and kinetic properties of events occurring there be measured correctly. In our data of the passive membrane properties of LD neurones and I–V relationships of the evoked response we did not find any indication of ineffective space clamping. In this respect this is completely different from recent data about clamping in slices of midbrain neurones (DeFazio & Walsh, 1996), in which no adequate dendritic voltage control could be achieved. The difference may depend on the morphological features of the corresponding neurones: the very thin dendritic arborizations in midbrain cells and the bulky dendritic tree in thalamic associative neurones.

The differences described in kinetic properties and expression of both types of LVA Ca^{2+} channels may indicate corresponding differences in their functional role. The role of Ca^{2+} currents generated by the 'fast' LVA channels may correspond to a similar (although still not quite clear) role of these currents in many other excitable cells, in which such channels appear for a short period during early ontogenesis. Obviously, they can already generate substantial Ca^{2+} transients during very weak excitatory inputs, which may be important for triggering the morphogenesis of the cell. This suggestion has been verified in experiments on DRG neurones clamped by digitally constructed waveforms that simulate natural action potentials, with photometric determination of induced Ca^{2+} transients (McCobb & Beam, 1991). These measurements have shown that Ca^{2+} entry through LVA channels forms a disproportionately large fraction of the total Ca^{2+} entry (40–50%) during brief action potentials. The entry through HVA Ca^{2+} channels increased more essentially as spike duration increased.

In contrast, 'slow' LVA Ca^{2+} currents are constantly present only in certain types of mature cells, and are obviously responsible for some specific types of their functional activity. Extensive analysis, using computer simulation, has already shown that they may be important for triggering periodic (pacemaker) and bursting neuronal activity, especially in conjunction with the presence of inhibitory (hyperpolarizing) synaptic inputs, releasing the corresponding channel from steady-state inactivation (Dossi, Nuñez & Steriade, 1992; Huguenard & Prince, 1992; Kang & Kitai, 1993; Hutcheon, Miura, Yarom & Puil, 1994). In some cases it has been shown that in adult neurones the LVA Ca^{2+} currents are confined to dendrites, and after their removal these currents disappear completely (Müller, Misgeld & Swandulla, 1992; Karst, Joels & Wadman, 1993).

The specific functional role of 'slow' LVA Ca^{2+} channels in thalamic associative neurones may be most important in pathological conditions, being involved in the generation of sensory epileptiform activity (Huguenard & Prince, 1992).

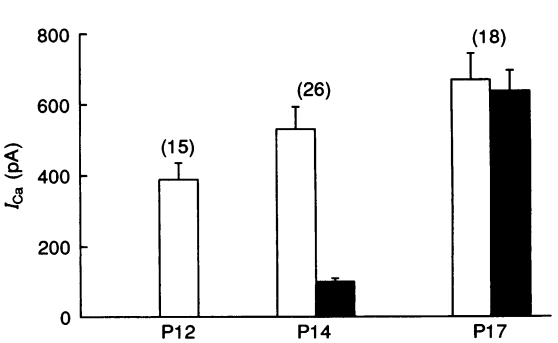

Figure 7. Age-dependent changes of the two components of the LVA Ca^{2+} current

The mean amplitudes of the $I_{T,f}$ and $I_{T,s}$ components were plotted as a function of postnatal age. Bars represent mean amplitude + S.E.M. of $I_{T,f}$ (□) and $I_{T,s}$ (■) components. Note that the fast $I_{T,f}$ component was well expressed during the early postnatal period; the $I_{T,s}$ component appeared at the end of second week and increased progressively up to P17. The number of cells investigated is indicated in parentheses.

In this respect, specific pharmacological properties of these channels may be of extreme applied importance in the search for new antiepileptic agents.

The structural basis of both types of LVA Ca^{2+} channels is still unknown, since no corresponding α_1-subunit has been identified. An attempt to express the LVA Ca^{2+} channel in *Xenopus* oocytes by injecting total mRNA isolated from the rat thalamo-hypothalamic complex produced complicated results. The Ca^{2+} currents induced demonstrated $I-V$ relationships typical of LVA currents with very slow inactivation, resembling the inactivation of $I_{T,s}$. On the other hand, these currents could be effectively blocked by dihydropyridines and La^{3+} in the range of concentrations typical for $I_{T,f}$ (Dzhura, Naidenov, Lyubanova, Kostyuk & Shuba, 1996). This may indicate postexpressional changes induced in the oocyte by changes in subunit composition of the Ca^{2+} channels (addition of different β-subunits which might be critical in the determination of channel kinetics); on the other hand, it may indicate the existence of even more variants of the α_1-subunits responsible for the formation of LVA Ca^{2+} channels.

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