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Modulation of voltage-gated channel currents by harmaline and harmane

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1 Harmala alkaloids are endogenous substances, which are involved in neurodegenerative disorders such as M. Parkinson, but some of them also have neuroprotective effects in the nervous system.

2 While several sites of action at the cellular level (e.g. benzodiazepine receptors, 5-HT and $GABA_A$ receptors) have been identified, there is no report on how harmala alkaloids interact with voltage-gated membrane channels.

3 The aim of this study was to investigate the effects of harmaline and harmane on voltage-activated calcium- ($I_{Ca(V)}$), sodium- ($I_{Na(V)}$) and potassium ($I_{K(V)}$)-channel currents, using the whole-cell patch-clamp method with cultured dorsal root ganglion neurones of 3-week-old rats. Currents were elicited by voltage steps from the holding potential to different command potentials.

4 Harmaline and harmane reduced $I_{Ca(V)}$, $I_{Na(V)}$ and $I_{K(V)}$ concentration-dependent (10–500 μM) over the voltage range tested. $I_{Ca(V)}$ was reduced with an IC_{50} of 100.6 μM for harmaline and by a significantly lower concentration of 75.8 μM ($P<0.001$, *t*-test) for harmane. The Hill coefficient was close to 1. Threshold concentration was around 10 μM for both substances.

5 The steady state of inhibition of $I_{Ca(V)}$ by harmaline or harmane was reached within several minutes. The action was not use dependent and at least partly reversible.

6 It was mainly due to a reduction in the sustained calcium channel current ($I_{Ca(L+N)}$), while the transient voltage-gated calcium channel current ($I_{Ca(T)}$) was only partially affected.

7 We conclude that harmaline and harmane are modulators of $I_{Ca(V)}$ *in vitro*. This might be related to their neuroprotective effects.

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Abbreviations: BMI, Biomedical Instruments; CNS, central nervous system; DRG, dorsal root ganglion; F-12, Hams F-12 medium; $GABA_A$, γ-aminobutyric acid a receptor subtype; GHB, γ-hydroxybutyrate; harmaline, 1-methyl-β-carboline; harmane, 1-methyl-7-methoxy-3,4-dihydro-β-carboline; 5-HT, 5-hydroxytryptamin; 5-HT_{2A}, 5-hydroxytryptamin 2A receptor; 5-HT_{2C}, 5-hydroxytryptamin 2C receptor; $I_{Ca(L+N)}$, sustained voltage-gated calcium channel current; $I_{Ca(T)}$, transient voltage-gated calcium channel current; $I_{Ca(V)}$, voltage-gated calcium channel current; $I_{K(V)}$, voltage-gated potassium channel current; $I_{Na(V)}$, voltage-gated sodium channel current; IC_{50}, inhibitory concentration reducing 50%; *I–V* curve, current–voltage relation curve; MAO, monoaminoxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin; NHE, sodium–hydrogen exchange; PC-12, rat adrenal pheochromocytoma

Introduction

The β-carbolines 1-methyl-β-carboline (harmane), 1-methyl-7-methoxy-3,4-dihydro-β-carboline (harmaline) and 9H-pyrido[3,4-b]indole (norharmane) are naturally present in the human food chain and supposed to occur endogenously in normal body constituents. They are found in the blood plasma, heart, kidney, liver and also in brain tissue, where they have been proposed to be endogeneous ligands for benzodiazepine and imidazoline receptors (Rommelspacher *et al*., 1980; May *et al*., 1994; Hudson *et al*., 1999). Since high plasma levels of these compounds have been found in heavy smokers (Spijkerman *et al*., 2002), alcoholics (Rommelspacher *et al*., 1991), heroin-dependent humans (Stohler *et al*., 1996), patients with essential tremor (Louis *et al*., 2002) or Parkinson's disease (Kuhn *et al*., 1996), they are assumed to have a crucial role in the pathophysiology of various disorders of the CNS.

Speculations on the biological significance of β-carbolines comprised cytotoxic as well as neuroprotective properties. On the one hand, β-carbolines have been postulated to act as endogeneous neurotoxins because of their structural similarity to 1-methyl-4-phenyl-1-1,2,3,6-tetrahydropyridine (MPTP) (Albores *et al*., 1990), a compound which induced a parkinsonian-like syndrome in animals (Przedborski & Jackson-Lewis, 1998). Furthermore, harmaline has been shown to have a cytotoxic effect on PC-12 cells (Cobuzzi *et al*., 1994) and to cause degeneration of Purkinje cells in rat cerebellum. The latter finding may explain its tremor-evoking action (O'hearn & Molliver, 1993).

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On the other hand, and, more recently, β-carbolines have been shown to protect neurones against the excitotoxic effects of dopamine and glutamate (Maher & Davis, 1996). Accordingly, a protective role of β-carbolines in the pathophysiology of Parkinson's disease has been suggested. Increasing evidence also hints to a protective effect of elevated endogeneous β-carbolines on oxidative neuronal damage. For example, β-carbolines were shown to depress the dopamine- or 6-hydroxydopamine-induced brain mitochondrial and synaptosomal dysfunctions, and also the loss of viability in PC-12 cells through a scavenging action on reactive oxygen species and maintenance of reduced thiols (Kim *et al*., 2001).

Apart from actions on benzodiazepine and imidazoline receptors, other effects of β-carbolines have been identified at the cellular level. These include an activation of 5-HT_{2A} and 5-HT_{2C} receptors, a potent inhibition of synaptosomal γ-hydroxybutyrate (GHB) re-uptake (McCormick & Tunnicliff, 1998), and an impairment of monoamine oxidase enzymes (MAO) and of sodium–hydrogen exchange (NHE) (Glennon *et al*., 2000; Anderson *et al*., 2003).

It appears surprising that, despite these manifold effects of harmale alkaloids on various targets, little information is available about their action on neuronal sodium, potassium and calcium channel currents and calcium homeostasis (Shi *et al*., 2001). Especially calcium – entering through membrane channels – acts as a major second messenger, whose intracellular concentration is tightly regulated. De-regulation results in reduced functionality of neurones, and a permanently increased calcium concentration within the cell could also result in malfunction and even cell death. Therefore, it might be possible that the opposing effects of harmala alkaloids described above may involve Ca_i^{2+}-homeostasis disturbances. In neurones, calcium enters the cell through different types of voltage-gated channels. The three main types of voltage-gated calcium channels which have originally been described and studied in dorsal root ganglion (DRG) neurones are L-, N- and T-type channels (Fox *et al*., 1987). They differ with respect to their voltage dependency and activation and inactivation properties (for details, see Hille, 1992). Most important for Ca_i^{2+} homeostasis are the noninactivating L-type channels, whose activation starts at a membrane potential of around −30 mV, and transient T-type channels, which are fast inactivating and open upon 'small' depolarisations (in the range of −50 to −10 mV). In this study, we investigated the effects of harmane and harmaline on voltage-gated calcium channel currents ($I_{Ca(V)}$) in comparison to sodium ($I_{Na(V)}$) and potassium ($I_{K(V)}$) channel currents. Using DRG neurones of adult rats and a concentration range of 10–500 μM harman or harmaline, we found that β-carbolines are indeed capable of reducing these channel currents.

A preliminary report has been published in abstract form (Splettstoesser *et al*., 2003).

Methods

Preparation of DRG neurones

DRG neurones were isolated from 3-week-old 'Wistar' rats. Animals were deeply anaesthetised with Isofluran (Curamed), until punching the rat tail and feet revealed complete analgesia. Thereafter, the rats were decapitated and the vertebral column was opened by a dorsal approach, starting at the caudal end. Spinal cord was removed and dorsal root ganglia (DRG) were collected by fine forceps from both sides of the spinal column and transferred into ice-cooled F-12 medium (Sigma, Taufkirchen, Germany). Spinal nerves were cut off under optical control using fine scissors. Then, the ganglia were transferred into a mixture of 0.9 ml F12 medium plus 0.1 ml collagenase medium (2612.5 U ml^{-1}, Sigma Type II) and digested in a humidified atmosphere (5% CO_2) at 37°C for 45–55 min. In the next step, the collagenase was removed by washing the ganglia with F-12 medium for at least three times. Trypsination (2525 U trypsin per ml F-12 medium, Sigma Type IX) was carried out for another 2–3 min under the same conditions. After adding F-12 medium (final volume 0.7 ml), the DRG were titurated with a fire-polished Pasteur pipette (tip diameter 150 μM) until the ganglion capsules were opened, and the neurones were released from the ganglia. A portion of 50 μl of the resulting suspension was placed in the middle of small petri dishes (3 cm; Falcon 'Easy Grip') and incubated for 2–4 h, allowing cells to adhere. Thereafter, 1 ml F-12 (with horse serum) was added to each petri dish. Cultures were used for electrophysiological recordings within the next 2 days.

Recording techniques and isolation of the different channel currents

With the whole-cell patch-clamp technique, membrane currents of DRG neurones were recorded using a HEKA EPC 9 amplifier and EPC screen software (HEKA). Microelectrodes were pulled from borosilicate glass with filament (o.d.: 1.5 mm and i.d.: 0.86 mm; Biomedical Instruments (BMI)) with a Sutter electrode puller (model P-87). Electrodes were fire polished with a Narashige micro forge (MF-830) to a final resistance of 3–4 MΩ and filled with the adequate internal solution (see Table 1; all chemicals for these solutions were obtained from Sigma-Aldrich, Taufkirchen, Germany). Special bath solutions were prepared to isolate the different types of voltage-activated channel currents (see Table 1). After a gigaohm seal had been obtained and a stable access to the neurones had been established, membrane potential was routinely clamped at −80 mV. Voltage-activated channel currents were elicited by depolarising command pulses to 0 mV for 120 ms (80 ms when measuring current–voltage (*I–V*) relations) for $I_{Ca(V)}$, 15 ms for $I_{Na(V)}$ and 200 ms for $I_{K(V)}$. Data were sampled at 10 kHz and stored on hard disc. To obtain *I–V* relations (*I–V* curve), depolarisation steps were started at −80 mV and were increased stepwise by 10 mV, until a maximal depolarisation to +60 mV for calcium and potassium channel currents and +40 mV for sodium channel currents was reached.

Harmaline and harmane (Sigma, Taufkirchen, Germany) were freshly dissolved in the respective bath solution (Table 1) at a concentration of 1 mM. Final dilutions (10–500 μM) were made immediately before use. Drugs were applied by a bath application system. To achieve total exchange of the bath saline, a volume of 10 ml was flushed through the bath (bath volume 1 ml), with a continuous flow of 5 ml min^{-1}.

Data analysis

All currents were leak corrected on-line with a P/3 protocol and off-line normalised to the mean of five successive peak currents (−80 to 0 mV) obtained under control conditions.

Table 1 Composition of external and internal solutions used to record ICa(V) (Calcium), INa(V) (Sodium) and IK(V) (Potassium)

	Calcium (mM)	*Sodium* (mM)	*Potassium* (mM)
External solutions			
NaCl		145	145
KCl		2.5	2.5
$MgCl_2$	1		1.2
$CaCl_2$			
$CdCl_2$			0.05
HEPES	10	10	10
TTX	0.001		0.002
4-AP			1
Glucose	10	10	10
$BaCl_2$	10		
TEA-chloride	130		
pH	7.3	7.4	7.4
Internal solutions			
CsCl	140	140	
NaCl		5	
$MgCl_2$	4	4	4
KCl			140
$CaCl_2$			1
HEPES	10	10	10
EGTA	10	10	11
Na-ATP	2		
pH	7.2	7.2	7.4

Calcium channel currents used for calculation of time-course, *I–V* relations and dose–response curves were rundown-corrected. Assuming a linear rundown, all current values were extrapolated to the time point of drug application.

To compare the mean inhibitory effects (inclusive the standard error) of harmaline and harmane on sodium, potassium and calcium channel currents over the voltage range tested, all currents were normalised and expressed as a percentage of the maximum current obtained over the voltage range tested. An *I–V* relation was drawn from these data, and the relative effect of both substances was calculated over the voltage range. Analysis of calcium channel currents comprised a separate evaluation of sustained currents (plateau at the end of the depolarisation) and the peak currents. To separate the transient peak current ($I_{Ca(T)}$), the underlying sustained current ($I_{Ca(L+N)}$) was subtracted from the total peak current ($I_{Ca(V)}$). With this, the specific influence of harmaline and harmane on the transient current became evident.

Dose–response curves were obtained by calculating the mean percentage of action of the normalised data as well as the standard error for each concentration of harmaline and harmane. Data were fitted using the Langmuir equation: $y = sc^h/(k^h + c^h)$, where c is the concentration, s the saturation (here 100), k the concentration at half-saturation and h the Hill coefficient.

Results

Both harmaline and harmane reduced the currents through voltage-activated calcium, sodium and potassium channels, but differed in efficiency and voltage dependence. The $I_{Ca(L+N)}$ (sustained current of voltage-gated calcium channels) was reduced most efficiently, when activating the channels by a depolarisation to 0 mV. $I_{Na(V)}$ (voltage-gated sodium channel currents) and $I_{K(V)}$ (voltage-gated potassium channel currents) were less affected by both substances when the channels were opened by the same depolarisation. Figure 1 illustrates the action of harmaline (a, c, e) and harmane (b, d, f) on voltage-activated calcium (a, b), sodium (c, d) and potassium channel currents (e, f) under control conditions and after a steady state of the effect had been reached. Raw traces of control currents and the currents after the application of 100 μM harmaline or 100 μM harmane are superimposed. Under control conditions, $I_{Ca(V)}$ (a, b) reached a peak within 4.9 ms and declined by $30 \pm 5\%$ over the period of activation (80 ms). Harmane was more effective in reducing the peak of the currents of these channels than harmaline, as it reduced these $I_{Ca(V)}$ currents by $56.2 \pm 0.5\%$, while harmaline blocked the current by $37.1 \pm 6.8\%$. $I_{K(V)}$, also activated by a depolarisation to ± 0 mV for 200 ms, were only weakly affected by harmaline or harmane (100 μM, each) (Figure 1e, f). This was also true for the $I_{Na(V)}$, elicited by a voltage jump to ± 0 mV for 15 ms (Figure 1c, d).

Dose–response relationship

We next investigated the dose–response relationship for the peak currents through calcium channels elicited by a depolarisation from the holding potential of -80 to ± 0 mV under the influence of harmane or harmaline (Figure 2). Fitting all data points to the Langmuir equation, we found a threshold concentration for inhibiting the currents being lower than 10 μM. Total inhibition ($>80\%$) was reached with concentrations above 250 μM for both substances. The IC_{50} value was calculated to be 75.8 ± 1.1 μM for harmane and 100.6 ± 5.3 μM for harmaline. The difference of 24.6 ± 2.4 μM was highly significant ($P<0.001$, paired *t*-test). The Hill coefficient was close to 1 under all conditions, indicating a single binding site for either of the substances.

Higher concentrations of harmaline or harmane were needed to reduce the $I_{Na(V)}$ and $I_{K(V)}$. A concentration of 100 μM harmaline reduced the $I_{Na(V)}$ by $23.0 \pm 5.8\%$ and $I_{K(V)}$ by $4.4 \pm 9.3\%$. A concentration of 500 μM blocked these currents by 75.4 ± 3.1 and $28.1 \pm 2.3\%$, respectively. Similar results were obtained using 100 μM (500 μM) of harmane: the $I_{Na(V)}$ was reduced by 4.2 ± 1.7 ($78.8 \pm 1.7\%$) and the $I_{K(V)}$ by 17.5 ± 5.0 ($75.1 \pm 5.7\%$). From this comparison, it became clear that harmaline and harmane most efficiently inhibited $I_{Ca(V)}$.

Voltage dependence of the effect

$I_{Ca(V)}$ (Figure 3a, b), $I_{Na(V)}$ (Figure 3c, d) and $I_{K(V)}$ (Figure 3e, f) were differentially affected by harmaline (Figure 3a, c, e) or harmane (Figure 3b, d, f) (with 100 μM each) over the voltage range tested.

Further analysis of the effects of both substances on the maximum current through $I_{Ca(V)}$ showed that the channel currents were reduced by 40–80% over the entire voltage range from -60 to $+40$ mV (Figures 3a, b and 4a, b).

A concentration of 100 μM of either substance revealed no obvious voltage dependence on $I_{Na(V)}$, while harmaline was a little more effective in reducing the current (Figure 3c, d). There was also no clear difference in the effect of harmaline or harmane when 500 μM was applied (Figure 3c, d). In both cases, the reduction varied between 75 and 95% over the voltage range tested (Figure 4c, d).

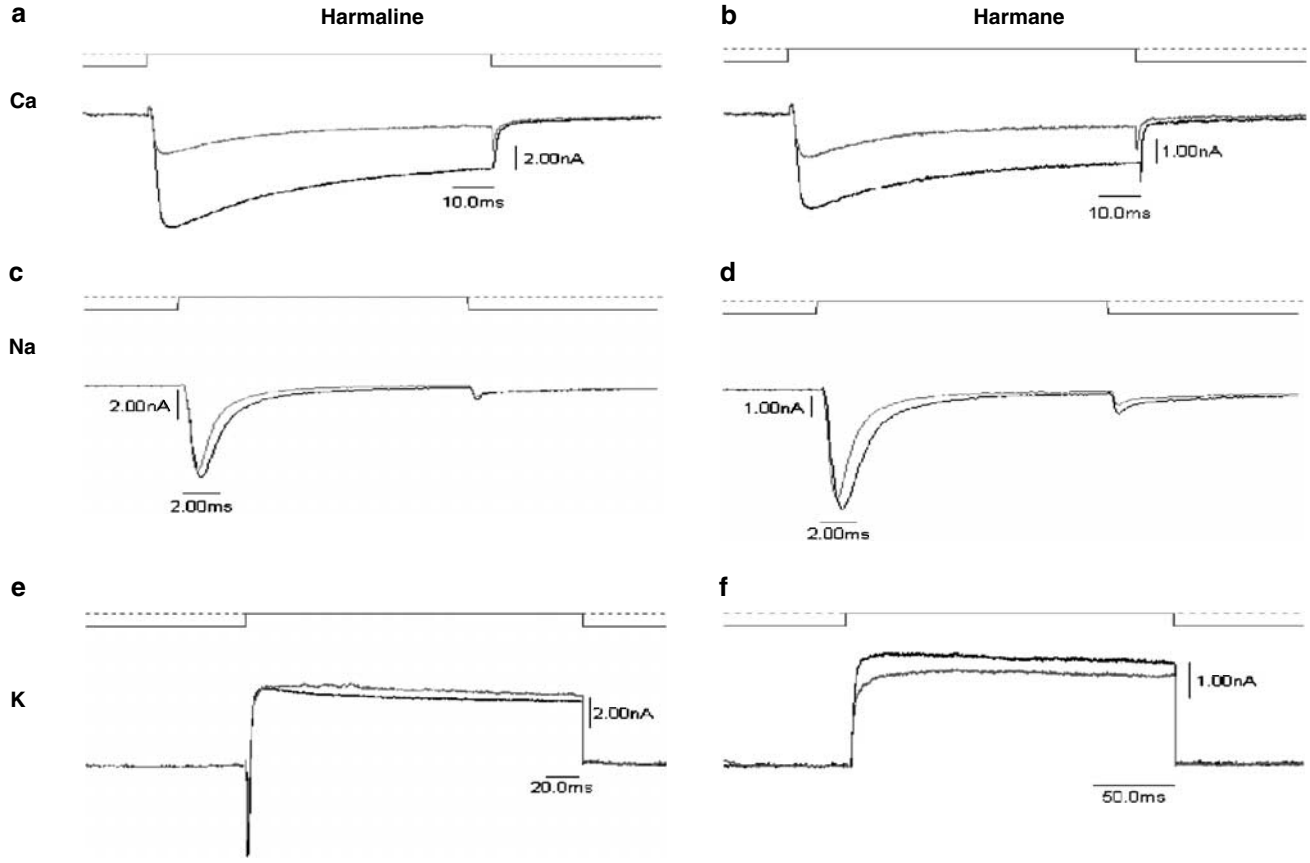

Figure 1 Raw traces of voltage-activated calcium (a, b), sodium (c, d) and potassium channel currents (e, f) elicited by a depolarisation from the holding potential of −80 to 0 mV (upper trace). Currents under control conditions (black; lower traces) and after blockade of the channel currents (grey) by 100 μM harmaline (a, c, e) or 100 μM harmane (b, d, f) are superimposed.

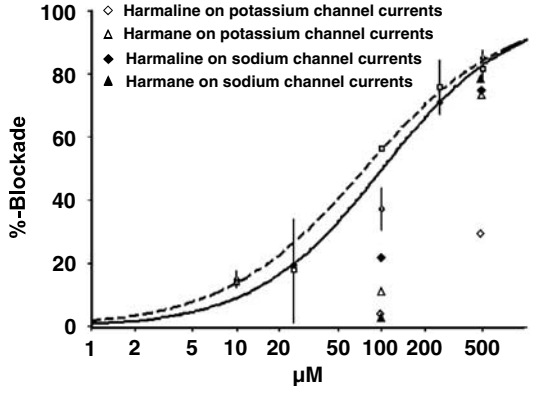

Figure 2 Dose–response relationship for the block of $I_{Ca(V)}$ by harmane (dashed line) and harmaline (black line) for a depolarising voltage jump from the holding potential of −80 to 0 mV for 100 ms. IC_{50}'s were 100.6 μM for harmaline and 75.8 μM for harmane. Threshold concentration was below 10 μM. For comparison, data for 100 and 500 μM harmane and harmaline on voltage-activated sodium and potassium channel currents are included (open diamonds are harmaline on potassium channel currents, open triangles are harmane on potassium channel currents, filled diamonds are harmaline on sodium currents and filled triangles are harmaline on sodium currents).

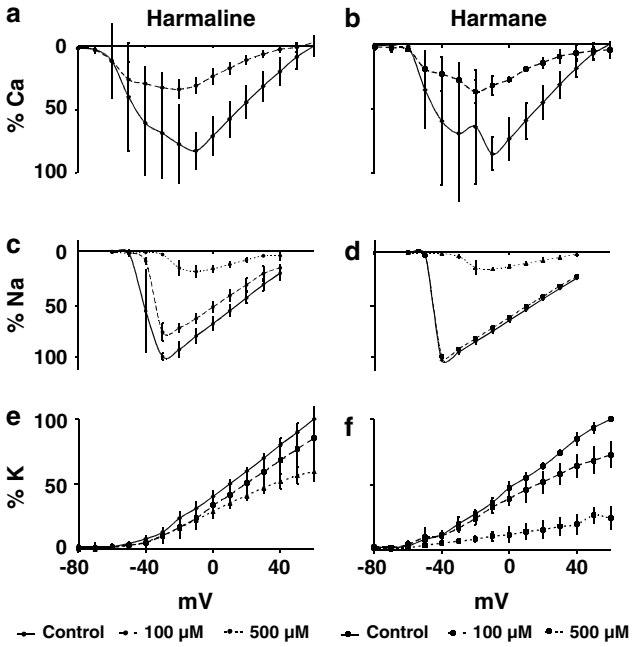

Figure 3 Normalised *I–V* relation of the effect of harmaline (a, c, e) and harmane (b, d, f) on $I_{Ca(V)}$ (a, b), $I_{Na(V)}$ (c, d) and $I_{K(V)}$ (e, f). Control *I–V*'s are represented by solid lines, effects of 100 μM of either substance are shown by dashed lines and the effects of 500 μM (for $I_{Na(V)}$ and for $I_{K(V)}$) by dotted lines.

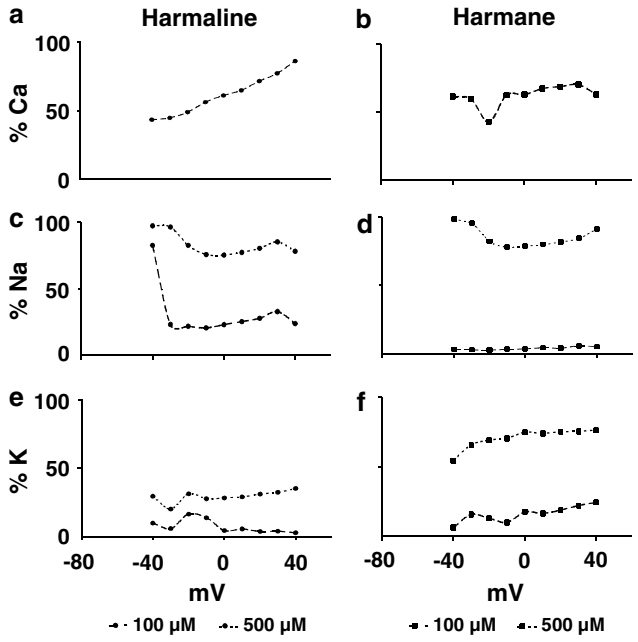

Figure 4 Relative reduction of $I_{Ca(V)}$ (a, b), $I_{Na(V)}$ (c, d) and $I_{K(V)}$ (e, f) by harmaline (a, c, e) and harmane (b, d, e) (100 or 500 μM each) over the voltage range tested. Data were calculated from the *I–V* relations as shown in Figure 3.

Similarly, the reduction of $I_{K(V)}$ lacked a clear voltage dependence (Figure 3e, f). At excessive concentrations (500 μM), the effect of harmane exceeded that of harmaline (Figures 2 and 3e, f). While harmaline reduced the current by 25% (at lower voltages) to 45% (at more depolarised voltages) (Figure 4e), the effect of harmane increased from 50% at lower to 75% at higher depolarisations (Figure 4f).

As both substances were most efficient in reducing the $I_{Ca(V)}$, in further experiments we focused on effects at these channels.

Time-course and use dependency of $I_{Ca(V)}$

The time courses of reducing the peak $I_{Ca(V)}$ by harmane or harmaline were not different. After application of either of the two drugs, a new lowered steady state of the reduction of the channel current was reached within 4–6 min (Figure 5a, b) subsequent to 25 and 100 μM harmaline (a) or harmane (b), respectively. This inhibition of $I_{Ca(V)}$ was reversible (up to 85% of control values) with a similar time course (Figure 5) of recovery.

To test whether an open-channel state is necessary for the action of harmaline (a) and harmane (b), calcium channel activation was commenced 5 min after application of the substances. Figure 6 shows that the normalised peak channel currents, activated by a depolarisation to $\pm$0 mV, had reached a clear steady state and showed no further decline while the substances were still present (Figure 6). This indicates that neither of the two substances needed an open-channel state to reduce the $I_{Ca(V)}$. As demonstrated in Figure 4, currents recovered were very similar during washout of the substances.

Differential effects on $I_{Ca(L+N)}$ *and* $I_{Ca(T)}$

The total peak channel current ($I_{Ca(V)}$) is composed of transient ($I_{Ca(T)}$) and sustained ($I_{Ca(L+N)}$) currents. While the sustained

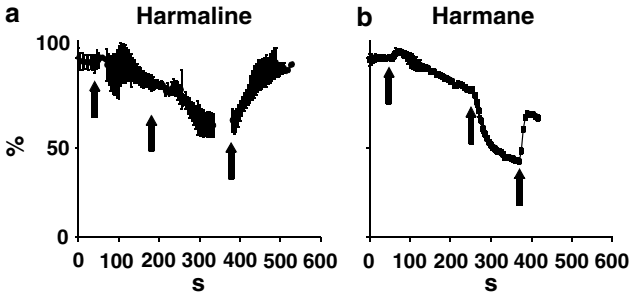

Figure 5 Averaged time course with standard deviation of the effect of harmaline (a; $n=8$) and harmane (b; $n=4$) on peak $I_{Ca(V)}$. Currents were elicited by a depolarisation from the holding potential of -80 to 0 mV. The first and second arrows in both panels mark the application of 25 and 100 μM of either substance, respectively. The third arrow indicates the beginning of wash-out of harmaline of harmane.

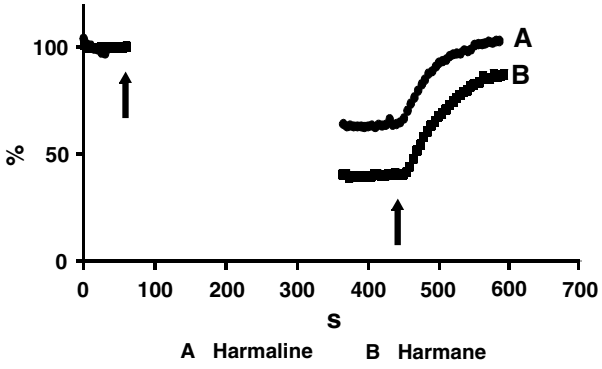

Figure 6 Absence of a use-dependent reduction of $I_{Ca(V)}$ by harmaline (A; 25 μM) and harmane (B; 100 μM). The voltage-driven activation of channel was interrupted for several minutes, while substances were applied (first arrow). When the activation was initiated after 350 s, a stable steady state had already been reached. Withdrawal of drugs (second arrow) resulted in a recovery of $I_{Ca(V)}$.

current ($I_{Ca(L+N)}$) at the end of the voltage jump (at 95 ms by depolarising to 0 mV) was largely reduced, there were only minor effects on the peak channel current ($I_{Ca(T)}$), indicating a different effect on these channels (Figure 1a, b).

This observation is underlined by the *I–V* relation. The $I_{Ca(L+N)}$ was plotted over the whole voltage range before and after the application of harmaline and harmane (Figure 7a, b). Clearly, in the voltage range between -10 and $+40$ mV, the reduction of the current varies between 60 and up to 100% for more depolarised voltages. This is about 5–20% above the reduction of the total peak current, as shown in Figure 4(a, b), and further indicates that the $I_{Ca(L+N)}$ is reduced to a degree higher than $I_{Ca(T)}$. To isolate the transient component ($I_{Ca(T)}$) from the total calcium channel current ($I_{Ca(V)}$), we subtracted the $I_{Ca(L+N)}$ from the total peak current. There was no clear indication for a reduction of this transient part of the current over most parts of the voltage range (Figure 7c, d). Therefore, the apparent reduction of the total peak current could be attributed mainly to the sustained component of the channel current.

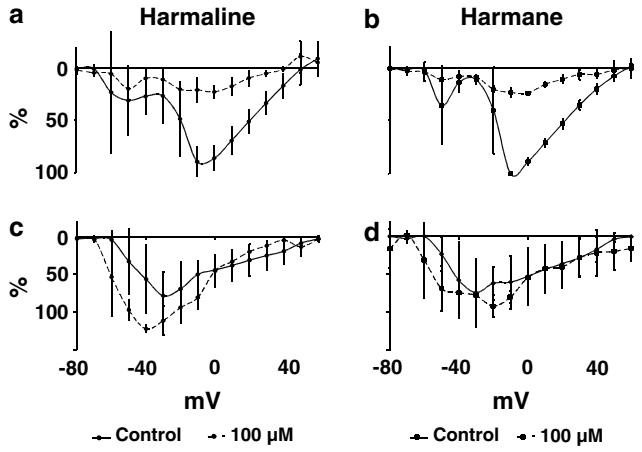

Figure 7 *I–V* relation for the sustained calcium channel current ($I_{Ca(L+N)}$), taken at the end of the depolarisation (a, b) and for the calculated transient peak current $I_{Ca(T)}$ (c, d). Control curves (solid lines) and of the relative effect of harmaline (a, c) and harmane (b, d), both 100 μM (broken lines), are shown. Note that sustained and calculated peak voltage-activated calcium channel currents were differentially affected. While the sustained current was maximally reduced at depolarised voltages above −10 mV, the calculated peak current was mainly unaffected over the voltage range tested.

Discussion

Harmaline and harmane reduce $I_{Ca(V)}$ voltage-activated calcium, sodium and potassium channel currents in rat DRG neurones over a wide voltage range and in a dose-dependent manner. Among these currents, $I_{Ca(L+N)}$ turned out to be the most sensitive, being inhibited by 50% using 100.6 μM harmaline or 75.8 μM harmane, respectively. A concentration of 100 μM of either substance reduced the $I_{Na(V)}$ only by 23.0±5.8 and 4.2±1.7%, respectively, and the $I_{K(V)}$ by 4.4±9.3 or 17.5±5.0% for a voltage step to 0 mV. For both substances, the effect on $I_{Ca(V)}$ was reversible and was not use dependent. Furthermore, there was a clear specificity in reducing the sustained calcium channel current (L-/N-type calcium channel), while the transient current (T-type channel) was not significantly affected. This is partially supported by the findings of Shi *et al*. (2000; 2001), who used binding receptor assays to demonstrate an interaction of the related compound harmine with the 1,4-dihydropyridine-binding site of L-type calcium channels of cardiac cells.

While neurotoxicity of harmaline has been discussed in the literature over several years (O'Hearn & Molliver, 1993; Cobuzzi *et al*., 1994), neuroprotective effects of harmaline and harmane have also been described (Bonnet *et al*., 2000). Their possible mechanisms, however, remained uncertain. Our results may shed some light on the underlying mechanisms and at least two explanations shall be proposed: (1) reducing voltage-activated sodium channels, and (2) reducing the current through voltage-gated calcium channels. Each of these mechanisms by itself causes a reduced excitation of neurones, which might result in limiting excitotoxicity. However, considering that such a reduction of $I_{Ca(V)}$ and $I_{Na(V)}$ occurs simultaneously, it is most likely that the effectiveness of possible neuroprotective efficiency of harmaline and harmane will even increase.

Although the reduction of voltage-activated sodium channel currents by harmaline and harmane occurred at high concentrations ($\geqslant$100 μM), inhibition became evident at relatively small depolarisations (starting at −40 mV). Therefore, the threshold for the generation of action potentials might be shifted to less negative values. This in turn would result in less action potentials and therefore in a reduced firing activity of neurones, as was also described by Carpentier (1982).

While the fast inactivating, transient calcium channel current (T-type current) was not significantly affected by both substances, the sustained current (L-/N-type current) was obviously more sensitive, with a threshold concentration for harmaline and harmane below 10 μM. Voltage-gated calcium channels of the L-/N-type – which are mainly carrying the sustained current – are localised primarily at the postsynaptic terminal of neurones (Igelmund *et al*., 1996). Thus, the increase in the calcium concentration within these compartments is certainly a major intracellular signal for numerous processes and neuronal function. Consequently, harmaline and harmane have the potency to interfere also with synaptic transmission.

Recently, harmala alkaloids were hypothesised to reduce apoptosis by inhibition of mitochondrial membrane-transition pores (Lee *et al*., 2000). Apototic or necrotic neuronal death can be triggered by an increase in the intracellular calcium concentration (Han *et al*., 2001), which might be prevented by harmaline or harmane. Hence, the reduced rise of intracellular calcium, putatively occurring under the influence of harmaline and harmane, will make programmed cell death less likely. But our technique focuses on the actions of voltage-gated membrane channel currents and does not allow to specify any intracellular changes of the calcium level resulting from the calcium release from intracellular stores.

In summary, harmaline and harmane are able to lower voltage-gated calcium channel currents at concentrations that are likely to be sufficient for neuro-protective effects *in vivo*. This mechanism is likely to contribute to changes in excitability owing to β-carboline components.

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