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The Effect of Captopril on the Membrane Properties of Central Neurons In-vitro

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Abstract—Captopril, a potent antihypertensive that acts via inhibition of angiotensin converting enzyme also has apparent central actions. Its effects on membrane properties on particular central neurons in-vitro has therefore been investigated. In the substantia nigra, where there is a high concentration of angiotensin converting enzyme, captopril caused a dose-dependent depolarization without any apparent change in conductance, but possibly requiring the integrity of the dendritic arbour. A similar effect occurred when captopril was applied to neurons in either the thalamus or hippocampus, where levels of angiotensin converting enzyme are relatively low. Further studies with homologues of captopril revealed that the −SH group on the molecule was a prerequisite of the effect observed. It is concluded that the −SH group on the captopril molecule has an electrogenic effect on diverse central neurons, independent of inhibition of angiotensin converting enzyme, but preferentially manifest at the level of the dendrite.

In 1976, an orally effective drug, captopril, was developed for hypertension to prevent accumulation of angiotensin II by inhibition of angiotensin converting enzyme (ACE) (Ondetti et al 1977; Cushman & Ondetti 1980). The drug was also thought to have potential psychiatric value (Zubensko & Nixon 1984; Etienne & Zubensko 1987). Although the neuronal mechanisms by which it appears to elevate mood are unknown, its selectivity for ACE inhibition could still be the proximate cause since ACE is present in the mammalian brain (Yang & Neff 1972), including that of man (Poth et al 1975).

The regional distribution of central ACE activity is sometimes (Printz et al 1982), but not necessarily (Yokosawa et al 1983) correlated with the renin-angiotensin system. The substantia nigra is a region where there is a paucity of angiotensin II or its receptors, but an extremely high level of ACE localized with [^{3}H]captopril binding (Strittmatter & Snyder 1987). It is conceivable, therefore, that central actions of the drug might be mediated via inhibition of ACE, which is exerting subsequent novel actions in nuclei such as the substantia nigra. The actions of captopril on the membrane properties of nigral neurons were therefore explored. The effects of the drug were also briefly studied in two other neuronal populations (hippocampus, thalamus) to see whether any potential effect of the drug on nigral cells was specific, and whether it was associated with a particular conductance of nigral cells, namely the low-threshold calcium spike, LTS gCa (Llinas et al 1984) which is also present in thalamus (Llinas & Jahnsen 1982), but not in hippocampus (Jahnsen 1986).

Materials and Methods

Preparation and maintenance of brain tissue in-vitro

Female albino guinea-pigs (ca 200g) were surgically anaesthetized with pentobarbitone (Sagatal, 2·0 mL i.p., 60 mg mL^{-1}) and decapitated. The brain was rapidly removed and coronal sections of mid-brain (400 μm) obtained as described previously (Llinas et al 1984; Nedergaard et al 1988a,b; Greenfield et al 1988). Typically, single slices of brain contained substantia nigra, thalamus and hippocampus. In all cases slices were maintained in a HEPES Ringer solution (see Nedergaard et al 1988a) for 2 h before the experiment. For recording, the tissue was subsequently submerged in a standard Ringer solution at 32°C (see below) with which it was perfused at a rate of approximately 2 mL min^{-1} by means of a peristaltic pump (see Harris et al 1989). In one series of experiments, the pars reticulata of the substantia nigra was sectioned as described by Harris et al (1989).

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Drug solutions and perfusates

All drugs were administered by perfusion in the standard Ringer solution (see Haas & Reiner 1988) containing (in mM): NaCl, 124; KCl, 2; KH_2PO_4, 1·3; $NaHCO_3$, 26; $CaCl_2$, 2·4; $MgSO_4$, 1·3; Glucose, 10. In one series of experiments calcium-free perfusate was used. In these experiments, to prevent precipitation of the divalent cations, the tissue was first perfused with a 'control' Ringer solution containing (in mM): NaCl, 137; KCl, 3·1; $NaHCO_3$, 26; $CaCl_2$, 2·4; $MgCl_2$, 2·0; Glucose, 10·7. Additional $MgCl_2$ was then substituted for $CaCl_2$ in this control solution (see Llinas & Sugimori 1980). All solutions were bubbled continuously with a 95% O_2–5% CO_2 gas mixture.

Captopril was dissolved in the standard Ringer solution in a concentration range of 10^{-6} to 10^{-2} M: this solution was adjusted with NaOH (1 M) to a final pH of 7·4. Other forms of the drug used were: the optical isomer *S*-captopril (SQ 14,534); the dimer captopril disulfide (SQ 14,551); and SQ 14,551 that had been cleaved by oxidation with formic acid such that the −SH group was replaced with $-SO_3H$.

Other drug solutions used were: tetrodotoxin (TTX), 300–600 nM; tetraethylammonium (TEA), 10 mM; β-mercaptoethanol, 1 mM; ascorbate, 1 mM.

Analysis of results

Values of resting membrane potential and input resistance were derived as described by Nedergaard et al (1988a). To

visualize particular active membrane properties, i.e. fast voltage transients, data were stored in a digitized form on a Neurodata IR-283 video recorder (Neurodata Instruments, New York) and plotted on a Hewlett Packard X-Y plotter using specially designed software on a Nimbus X 16 microcomputer (Research Machines, Oxford). Statistical analyses between different groups of cells under various conditions employed an unpaired Student's *t*-test.

Results

Passive and active properties of cells recorded

All neurons used had a stable resting membrane potential for 30 min at least, before experiments were attempted. The mean membrane potential and input resistance ± s.e.m.s for the three neuronal populations were, respectively, substantia nigra: $-60{\cdot}7 \pm 1{\cdot}3$ mV, $83{\cdot}1 \pm 8{\cdot}5$ Mohms, n = 32; thalamus: $-65{\cdot}8 \pm 4{\cdot}9$ mV, $88{\cdot}8 \pm 18{\cdot}5$ Mohms, n = 4; hippocampus: $-68{\cdot}3 \pm 6{\cdot}9$ mV, $88{\cdot}7 \pm 22{\cdot}2$ Mohms, n = 3. All three types of cell displayed a characteristic shape of action potential as described previously, and respectively, for substantia nigra, pars compacta (Llinas et al 1984), hippocampus, CA1 (Schwartzkroin 1977) and thalamus, lateral geniculate nucleus (Llinas & Jahnsen 1982). Despite similarities in mean membrane potentials and input resistances, all three types of cell frequently displayed differences. The mean firing frequency of substantia nigra neurons was $1{\cdot}8 \pm 0{\cdot}3$ impulses s^{-1}, whereas thalamic neurons were quiescent. Of the three hippocampal neurons examined, two were quiescent and one was spontaneously active, firing 6·0 impulses s^{-1}. In some, but not all nigral cells, termination of a hyperpolarizing current injection resulted in synchronous action potentials (Fig. 1Ai). This was not the case in the spontaneously firing hippocampal cell (Fig. 1Ci). In thalamic cells, larger negative current injections often resulted in burst firing (Fig. 1Bi).

Despite differences in firing rates and patterns, the three cell types were similar in certain conductances. Eighty percent of substantia nigra, and all hippocampal neurons displayed inward 'anomalous' rectification during large negative current pulse injections (see Fig. 1A, C). Of the four thalamic neurons examined, three displayed inward rectification (see Llinas & Jahnsen 1982). All three cell groups displayed slow depolarizations which differed in duration and amplitude (see Fig. 1Aii, Bii, Cii). These slow depolarizations seen in recorded nigral and thalamic cells, respectively, are similar in appearance to the low threshold calcium spikes 'LTSgCa', which are characteristic of these cell types (Llinas & Jahnsen 1982; Llinas et al 1984). The small residual depolarization seen in hippocampal cells may also be a calcium-dependent conductance, although the presence of an LTS in these cells has not previously been recorded in the literature (Jahnsen 1986).

In three cases where the pars reticulata of the substantia nigra was severed, no LTS was observed (see Harris et al 1989): the mean membrane potential of these cells was $-58{\cdot}3 \pm 4{\cdot}6$ mV and the input resistance, $50{\cdot}3 \pm 4{\cdot}9$ Mohms. Two of these cells were quiescent and one was spontaneously firing at 3·0 impulses s^{-1}.

Effects of captopril in the substantia nigra

Captopril caused a dose-dependent depolarization in nigral neurons in concentrations of 10^{-5} M and higher (see Fig. 2). However, this change in membrane potential was not accompanied by any change in input resistance nor in any discernible excitability in the cells. When captopril (10^{-2} M) was perfused through the bath in the absence of a brain slice, there was no voltage deflection due to a change in the electrode tip potential. In three cases where the pars reticulata was sectioned, the effect of captopril was significantly ($P < 0{\cdot}001$) reduced (Fig. 3A).

Application of TEA resulted in an increase in input resistance and a modification in the shape of the action potential consistent with a blockade of potassium channels. In four cells, captopril administration at a mean dose of $0{\cdot}8 \pm 0{\cdot}2$ mM had a more marked effect in the presence of

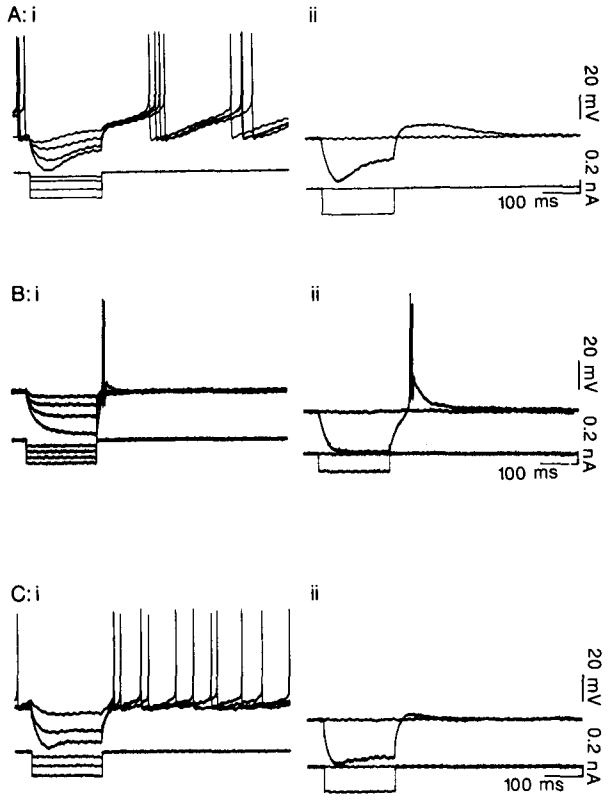

FIG. 1. Diverse membrane properties displayed by three groups of central neurons during similar recording procedures.

(A) *Substantia nigra.* Termination of a negative current injection leads to generation of synchronous action potentials (i). However, when the cell is hyperpolarized by about 15 mV from the resting membrane potential of −63 mV, repolarization leads to a sustained depolarization of approximately 300 ms in duration and 10 mV in amplitude (ii). Note the pronounced inward rectification during hyperpolarizing pulse (i,ii).

(B) *Thalamus.* Termination of a negative current injection finally leads to burst firing at 0·4 nA (i). However, when the cell is hyperpolarized by approximately 15 mV from the resting membrane potential of −57 mV, repolarization leads again to burst firing but generated from a more pronounced slow depolarization of 150 ms duration and 20 mV amplitude (ii).

(C) *Hippocampus.* Termination of a negative current injection leads to no apparent change in either the firing rate or pattern of the cell, which was firing repetitively at 6 Hz (i). However, when the cell is hyperpolarized by approximately 15 mV from the resting membrane potential of −56 mV, repolarization leads to generation of a small, residual depolarization of approximately 50 ms duration and 5 mV amplitude. Note the small degree of inward rectification during hyperpolarizing pulse (i, ii).

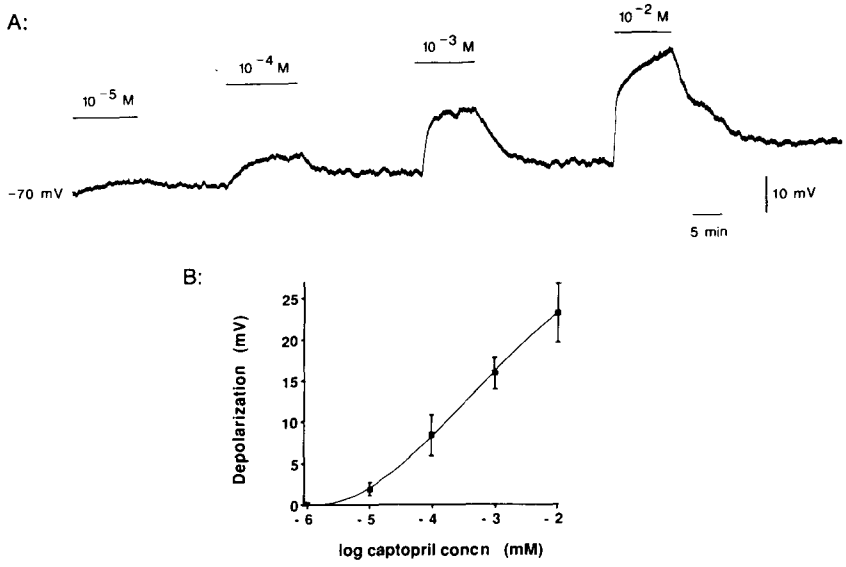

FIG. 2. Dose-dependent effects of captopril in the substantia nigra.
(A) The resting membrane potential of a single pars compacta neuron before, during and after captopril application (denoted by the horizontal bars). Note that there is a dose-dependent depolarization in response to captopril.
(B) Dose-response curve for the depolarizing actions of captopril. Values show mean depolarization (mV) ± s.e.m. for each dose up to 10^{-2} M. The total number of applications (n) for each dose is as follows: 10^{-6} M, 6; 10^{-5} M, 6; 10^{-4} M, 12; 10^{-3} M, 23; 10^{-2} M, 10.

TEA, which was significant at the level $P < 0.05$. However, in three other cells which had similar membrane properties, the depolarization was not significantly different from control conditions following a similar mean dose of 0·7 ± 0·3 mM (Fig. 3B). In the absence of TEA, the control response of the TEA-sensitive cells to captopril (14·8 ± 2·0 mV) was significantly lower than that of the TEA-insensitive cells (23·7 ± 0·9 mV) at the level $P < 0.02$. The magnitude of the effect of captopril did not relate to specific passive or active membrane properties (see Llinas et al 1984) of the neuron. Furthermore, the effect was resistant to sodium channel blockade with TTX (n = 3) and to removal of calcium from the perfusing medium (n = 3).

Effects of captopril homologues in the substantia nigra

Captopril disulfide appeared to have no effect on the membrane properties of nigral neurons (Fig. 4). On the other hand, *R,S*-captopril caused a change in membrane potential indistinguishable from that seen following application of captopril itself (Fig. 4). However, when the −SH group was modified on the captopril molecule, the resulting compound was without effect (Fig. 4). β-Mercaptoethanol and ascorbate application in both cases caused a depolarization similar to that seen for captopril in standard conditions (mean depolarization 14·0 ± 2·9 mV, n = 3; 13·3 ± 3·4 mV, n = 3, respectively).

Effects of captopril in the thalamus and hippocampus

When captopril was applied to neurons in either the thalamus or hippocampus, there was a dose-dependent depolarization with no apparent change in conductance, reminiscent of the effect seen following administration of the drug in the substantia nigra. Indeed, there was no significant difference in the amplitude of the effects of captopril in all three areas (cf. Fig. 5 and Fig. 2).

Discussion

Effects of captopril in the substantia nigra

Administration of captopril caused a dose-dependent depolarization, but without any apparent change in neuronal input resistance or excitability, i.e. in overall conductance. Nonetheless, its effect would appear to be a true transmembrane event since it was ensured that the pH of the drug solution did not differ from control perfusate and that the captopril itself did not have a direct effect on the tip potential of the electrode.

It could be argued that the concentrations of captopril used were relatively high compared with clinically-administered doses. However, it would be difficult, if not impossible, to ascertain the exact extracellular concentrations of the chronically administered drug in human substantia nigra in-vivo. Furthermore, it has been shown that, in the highly artificial brain slice preparation, the concentration of a substance falls by a factor of ten for every 60 μm depth increment of the recording site from the cut surface. It is possible therefore that the concentration of captopril at the site of recording is much lower than the bath concentration (Brown & Scholfield 1984).

A possible explanation for the voltage drop observed might be that at least two ions were involved which had equal and opposite effects on membrane conductance, such that no net change in resistance was detectable. However, were this to be so, modification of the flux of one of the three most common ions with TTX, TEA or Ca^{2+}-free perfusate should have unmasked a captopril-induced change in conductance. Yet, captopril did not modify input resistance in addition to membrane potential following any of these manipulations. This observation regarding Ca^{2+}-free medium and TTX would also discount the hypothesis that the effect of captopril on potential but not resistance, was mediated by a

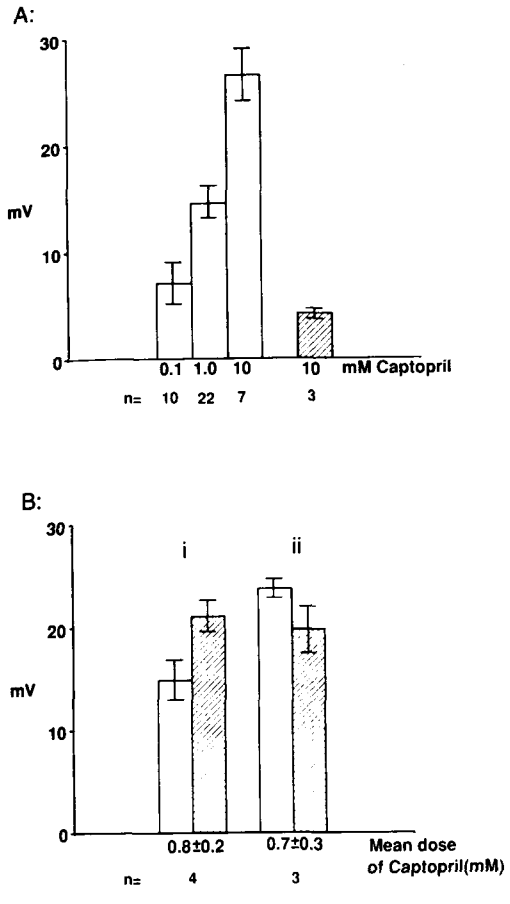

FIG. 3. Modifications to the effect of captopril.

(A) Histogram showing effects of sectioning the pars reticulata on the efficacy of captopril administration. First three values (open column) show dose-dependent depolarizing effects of captopril for n cells. However, fourth value (hatched column) shows effects of a high dose of captopril (10 mM) on three cells where the pars reticulata had been removed. This value differs from an equivalent dose in control neurons at the level $P < 0{\cdot}001$.

(B) Histogram showing the response to captopril in two apparently similar populations of nigral neurons (i and ii), which were distinguishable by the effects of TEA (10 mM) on their response. In control conditions (open column), comparable mean doses of captopril produced depolarizations that were significantly different from each other in amplitude at the level $P < 0{\cdot}02$. However, following TEA application (hatched column), the captopril effect was either significantly enhanced (i), at the level $P < 0{\cdot}05$, or was unchanged (ii).

single ion (i.e. Ca^{2+} or Na^+) with a reversal potential far from resting membrane potential (approximately +130 and +40mV, respectively, under our experimental conditions, T=32°C, $[Ca^{2+}]_o$ 2·4 mM, $[Na^+]_o$ 150 mM and assuming $[Ca^{2+}]_i$ 100 nM, $[Na^+]_i$ 30 mM).

However, another hypothesis to explain the effect of captopril is that the drug was acting at a site electrotonically remote from the soma. The modification of the effect of captopril by TEA would support this. By blocking resting potassium conductance, TEA improves the passive electrotonic properties of the neuron, such that events remote from the recording site (the soma) are more readily visualized. Indeed, in one population of cells, in the presence of TEA, captopril induced a larger effect than in control conditions. However, in an apparently similar cell population, a comparable dose of the drug induced a depolarization that was not significantly changed in the presence of TEA: this discrepancy could be explained as a form of tachyphylaxis, since the control response to captopril in the latter group was significantly larger initially. In any event it would seem that captopril might act preferentially at sites remote from the soma, i.e. the dendrites, since sectioning the pars reticulata, which contains large amounts of neuropil, attenuated the effect normally seen. Indeed, within the substantia nigra, captopril binding has been found to be most intense in the pars reticulata (Strittmatter & Snyder 1987). A further, and not mutually exclusive possibility for explaining the effect of captopril, is that it might have an electrogenic action, i.e. initiate a change in ionic flux without a conductance change, as has been postulated for glutamate uptake in retinal glial cells (Brew & Attwell 1987; Barbour et al 1988). The apparent favourable action of captopril on smaller surface areas (dendrites), would support this idea.

Mechanism of action of captopril

If captopril acts preferentially within the substantia nigra, the most parsimonious explanation would be that the action is via inhibition of ACE which is high in this region (Strittmatter & Snyder 1987; Chai et al 1987). However, *R,S*-captopril, which is one hundred times less potent an ACE inhibitor (Cushman et al 1977), displayed an identical action. The captopril disulfide dimer which also is not a significant ACE inhibitor (Drummer et al 1985), was inefficacious, as was the modified captopril in which the −SH group had been replaced with an $-SO_3H$ group. Captopril and *R,S*-captopril differ from captopril disulfide and the modified captopril in that the two former drugs possess a free −SH group. It is tempting to suggest that the −SH group is responsible for the effect of captopril since β-mercaptoethanol, which also contains an −SH group, induced comparable effects. Nonetheless, it seems unlikely that the −SH group served to chelate divalent cations, as the effect of captopril persisted in calcium-free medium. Perhaps the −SH group acted as a reducing agent: the totally unrelated reducing substance, ascorbate, has a similar effect.

Effects of captopril in thalamus and hippocampus

A further reason for not attributing the effect of captopril to inhibition of ACE is that the drug was equally effective in the thalamus and hippocampus, two brain regions where levels of ACE are negligible (see Strittmatter & Snyder 1987). Furthermore, even though neurons in all three areas were sensitive to captopril, there are no specific electrophysiological (see Fig. 1) or morphological features (see Grossman et al 1973; Rafols & Valverde 1973; Juraska et al 1977; Shepard 1979) in common to all three. Nonetheless, the results from the experiments in which the pars reticulata was sectioned, which were discussed earlier, have implicated the possible importance of the dendrites in the actions of captopril. Perhaps then, the significant feature common to all three cell populations studied, is the mere presence of dendrites, rather than specific characteristics of the dendritic arbours or the lengths of the dendrites themselves.

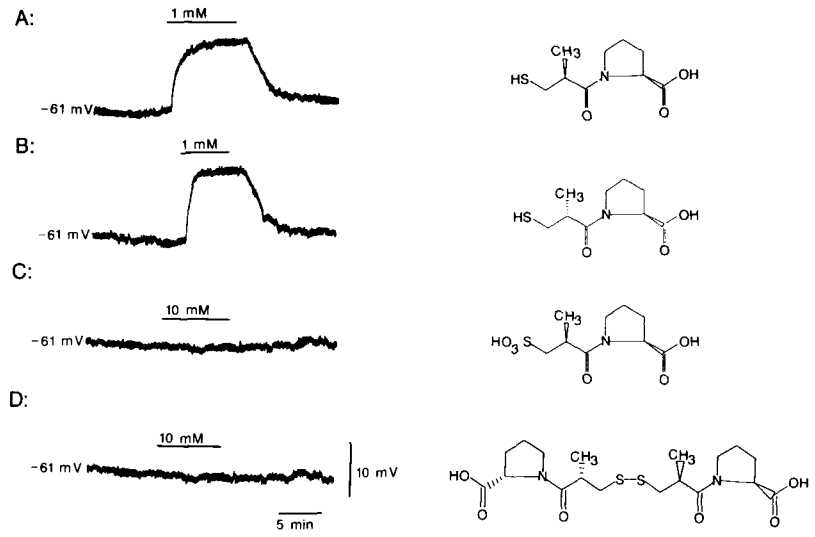

FIG. 4. Effects of various captopril homologues on a single pars compacta neuron in the substantia nigra.

A. The resting membrane potential of the neuron before, during and after 1 mM captopril application (as denoted by the horizontal bar) showing a depolarizing response. Note the chemical structure, as seen opposite, contains a free −SH group.

B. The resting membrane potential of the neuron before, during and after 1 mM *R,S*-captopril application (as denoted by the horizontal bar) showing an identical depolarizing response. The chemical structure of *R,S*-captopril is the same as that of captopril except for the orientation of the chiral carbon and, as seen opposite, also contains a free −SH group.

C. The resting membrane potential of the neuron before, during and after application of modified captopril (as denoted by the horizontal bar) at ten times the concentration. There is now no response. Note in the chemical structure opposite, that the −SH group has been replaced with an $-SO_3H$ group.

D. The resting membrane potential of the neuron before, during and after application of the dimer captopril disulphide (as denoted by the horizontal bar) at ten times the concentration. There is now no response. Note the absence of a free −SH group in the chemical structure.

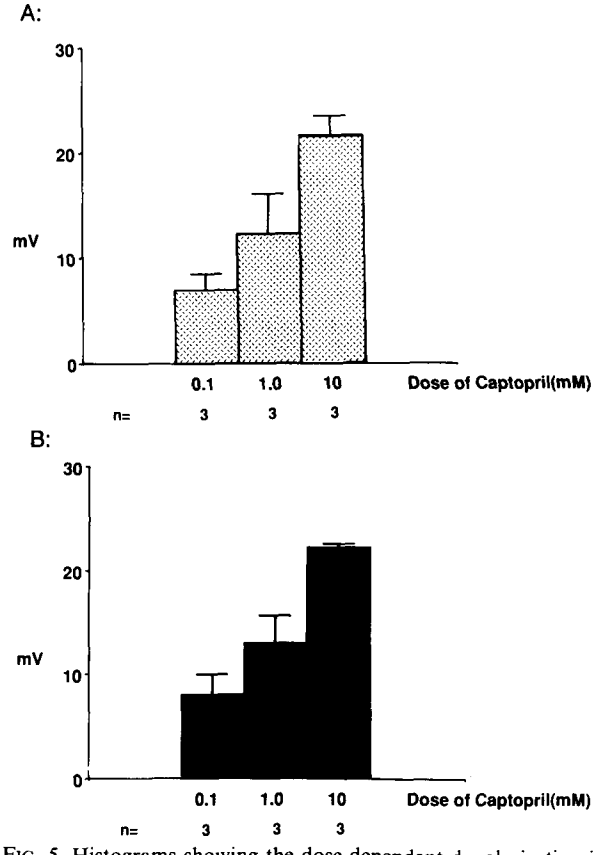

FIG. 5. Histograms showing the dose-dependent depolarization in response to captopril for four thalamic neurons (A) and three hippocampal neurons (B). The number of applications (n) for each dose are indicated.

Conclusions

It appears that captopril has an electrogenic action, possibly on neuronal processes with a small surface area (dendrites), independent of any particular dendritic conductance. The basic finding reported here is that captopril has caused a depolarization. However, this effect in itself tells us little of its mechanism of action since the net response of the cell membrane can only be a hyperpolarization or a depolarization irrespective of the particular experimental treatment. In this case, this depolarizing action is not due to ACE inhibition but is most readily attributed to the −SH group on the molecule. Subsequent experiments suggested that the −SH group acts here as a reducing agent.

It is interesting that despite the high level of binding of captopril in the substantia nigra, there appears to be no electrophysiological correlate of this regional selectivity. Indeed in the light of this study, it would seem unlikely that captopril-induced elevations in mood, for example, would be due to direct ACE inhibition within specific central nuclei.

Chronic oral therapy with captopril has been shown to increase significantly ACE activity in the medulla oblongata and mid-brain regions in the spontaneously hypertensive rat, suggesting that the drug may be able to cross the blood-brain barrier and that certain areas of the brain may be permeable to it (Di Nicolantonio et al 1984). Furthermore, there has been a report that captopril made the symptoms of Huntington's chorea worse in one patient (Goldblatt & Bryer 1987). Further work to examine the degree of captopril penetration into key brain areas and its subsequent effects on central neurons is thus of significant clinical relevance. In conclusion, the material presented here describes a novel

neuropharmacological property of a widely prescribed drug. This finding should therefore be of basic relevance to studies of captopril in the CNS.

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