Central action of dendrotoxin: Selective reduction of a transient K conductance in hippocampus and binding to localized acceptors

(K channels/facilitatory neurotoxin/neuronal binding protein)

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ABSTRACT   Dendrotoxin, a small single-chain protein from the venom of Dendroaspis angusticeps, is highly toxic following intracerebroventricular injection into rats. Voltage-clamp analysis of CA_1 neurons in hippocampal slices, treated with tetrodotoxin, revealed that nanomolar concentrations of dendrotoxin reduce selectively a transient, voltage-dependent K conductance. Epileptiform activity known to be induced by dendrotoxin can be attributed to such an action. Membrane currents not affected directly by the toxin include (i) Ca-activated K conductance; (ii) noninactivating voltage-dependent K conductance; (iii) inactivating and noninactivating Ca conductances; (iv) persistent inward (anomalous) rectifier current. Persistence of the effects of the toxin when Cd was included to suppress spontaneous transmitter release indicates a direct action on the neuronal membrane. Using biologically active, ^{125}I-labeled dendrotoxin, protein acceptor sites of high affinity were detected on cerebrocortical synaptosomal membranes and sections of rat brain. In hippocampus, toxin binding was shown autoradiographically to reside in synapse-rich and white matter regions, with lower levels in cell body layers. This acceptor is implicated in the action of toxin because its affinities for dendrotoxin congeners are proportional to their central neurotoxicities and potencies in reducing the transient, voltage-dependent K conductance.

Neurotransmitter release is facilitated at peripheral (1) and central (2, 3) synapses by a homologous group of single-chain neurotoxins (4), which are devoid of known enzymatic activity (1). Two of these mamba snake proteins, toxin I and dendrotoxin (DTX), from Dendroaspis polylepis and angusticeps, respectively, are highly neurotoxic when injected intracerebroventricularly into rats (5). Electrophysiological recordings in hippocampal slices from guinea pig or rat have demonstrated that DTX is a potent convulsant (2, 3) that causes enhancement of cell excitability with a concomitant potentiation of transmitter release. With the aim of elucidating the mechanism of action of these novel facilitatory polypeptides, we have studied their effects on a range of membrane currents in hippocampal neurons. Our findings indicate that the pronounced central toxicity and convulsive action of DTX can be attributed to a reduction of a transient K conductance [i.e., the transient, voltage-dependent K current I_A (6, 7)]. Moreover, a membrane acceptor protein with a high affinity for DTX was identified, and its discrete localization in hippocampus established. It is concluded that DTX is a useful pharmacological tool for studying voltage-sensitive K channels responsible for I_A, and variants of the latter.§

MATERIALS AND METHODS

Purification and Radioiodination of DTX. The neurotoxin was purified to homogeneity from the venom of D. angusticeps (Sigma), characterized as detailed (8) and ^{125}I-iodinated using a modification of the chloramine-T method that avoids loss of neurotoxicity (2).

Electrophysiological Recording in Hippocampal Slices. Methods for preparation and maintenance of brain slices from guinea pig or rat have been described (9, 10). DTX and 4-aminopyridine (4AP) were dissolved in Krebs medium and administered for required periods by superfusion at 28°C. Cells in the CA_1 region of the slice were impaled with glass micropipettes filled with 3 M KCl, 4 M KOAc or 3 M CsCl, giving dc resistance values of 50–80, <120, and 40 MΩ, respectively. Intracellular recordings were made by using a single electrode voltage-clamp amplifier (10). Measurements of current and voltage were routinely taken at the end of 0.5- or 1-sec clamp steps to obtain steady-state current–voltage relations (CVRs). For the construction of near-instantaneous CVRs, measurements were made at the earliest opportunity after the initiation of a voltage jump when the voltage had settled to within 1 mV of the steady-state command potential.

Assay of ^{125}I-Labeled DTX Binding to Synaptosomal Membranes and Brain Sections. Freeze-thawed synaptosomes, purified from rat cortex (8), were resuspended in Krebs-phosphate buffer, pH 7.4 (≈0.7 mg of protein/ml); where stated, Ca^{2+} was omitted and 1.3 mM EGTA included. Binding of ^{125}I-labeled DTX (^{125}I-DTX) was assayed in duplicate samples by rapid centrifugation of the membranes through oil (2, 8); nonspecific binding was determined likewise by adding 100-fold excess of DTX. For competition experiments, a standard centrifugation assay (11) was used where 2.5 nM ^{125}I-DTX was incubated as above with synaptosomes in the absence and presence of pure preparations (2, 11) of various proteins. ^{125}I-DTX binding to brain sections (see below) was performed by using tissue that was dry mounted onto slides; after labeling with toxin and washing, test and control specimens were detached from the slides prior to quantitation of ^{125}I-DTX bound by gamma counting.

Light-Microscope Autoradiography. Lightly fixed whole brain from rats that had been perfused with 0.1% paraformaldehyde/phosphate-buffered saline, pH 7.4 was used to cut cryostat serial sections (10 μm). After labeling with ^{125}I-DTX, the mounted sections were processed for autoradiography using LKB Ultrofilm (12).

Abbreviations: DTX, dendrotoxin; ^{125}I-DTX, ^{125}I-labeled DTX; TTX, tetrodotoxin; CVR, current–voltage relation; 4AP, 4-aminopyridine; I_A, transient K current; I_C, Ca-activated K current; I_M, a noninactivating time- and voltage-dependent K current, triggered by depolarizing the cell positive to around −60 mV; I_Q, a mixed Na/K current activated by hyperpolarization.
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RESULTS

Effect of DTX on Evoked and Spontaneous Activity in Hippocampal Neurons. Tetrodotoxin (TTX) was routinely administered to eliminate Na action potentials and, hence, simplify analysis of single neurons. Twenty-one cells in the CA_1 region were studied under current- or voltage-clamp conditions; control resting membrane potential was -68 ± 2 mV ($\pm$SEM), input-resistance was 60 ± 4 MΩ, and where measured before the addition of TTX, action potential amplitude was 96 ± 4 mV in the cells impaled with K-containing pipettes. The control in Fig. 1A shows the voltage responses of a neuron, in the presence of 0.5 μM TTX, to current injection. Depolarizing current elicited broad (presumed Ca-dependent) spikes; these were enhanced in number and evoked with lower current strengths after 1 hr exposure to 50 nM DTX (Fig. 1A), while little change in the responses to hyperpolarizing current or in the resting membrane potential was observed ($n = 3$). Similar alterations in Ca spikes were observed in six of seven other cells exposed to higher DTX concentrations (250–300 nM); these could be partially reversed by the addition of 300 μM Cd (a Ca-channel blocker), suggesting involvement of Ca channels in the depolarizing response.

DTX Does Not Affect Ca Channels Directly. In three neurons impaled with electrodes containing 3 M CsCl [which blocks outward K movement (15) and reveals Ca currents], DTX (250 nM) materially affected neither the transient Ca current (13) nor the sustained Ca current (Fig. 1B) (14). Such results eliminate the possibility that DTX directly enhances
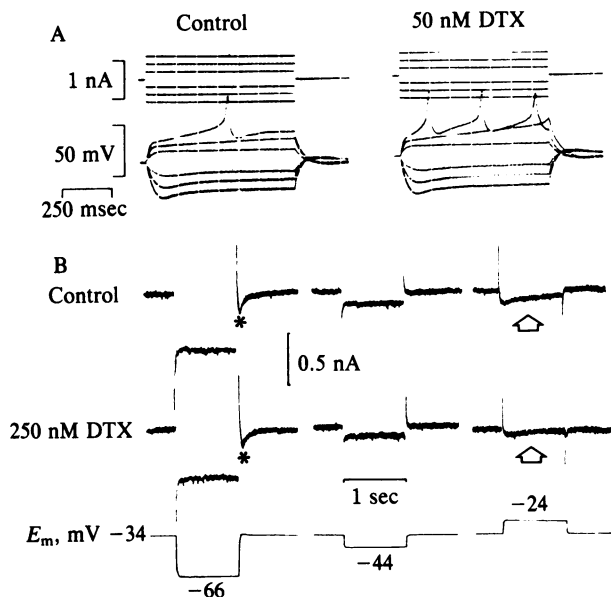

FIG. 1. Differential effects of DTX on Ca spikes and Ca currents in hippocampal neurons. (A) Voltage responses (lower traces) to intracellular current injection (upper traces) from a hippocampal CA_1 neuron impaled with a microelectrode containing 3 M KCl, and superfused with medium containing 0.5 μM TTX alone (control) or 0.5 μM TTX and 50 nM DTX for 1 hr. Resting potential in each case was -75 mV. Note that after DTX was administered, Ca spikes were elicited at lower current strengths and more repetitive firing was observed. (B) Transmembrane currents (upper two traces) driven by the voltage steps indicated (lower lines) from a holding potential of -34 mV in a cell impaled with a microelectrode containing 3 M CsCl under voltage clamp with the single electrode-clamp amplifier. Upon repolarization from -66 mV a transient inward Ca current (13) is elicited (*); stepping to -24 mV triggers a sustained Ca current (13, 14) (arrow). Note that neither current was potentiated after exposure to 250 nM DTX for 1 hr. TTX (0.5 μM) was present throughout.

Ca spikes by increasing flux through Ca channels responsible for the two Ca conductances described in these cells (13, 14).

Resistance of Other Currents to DTX. Possible action of DTX on three other identifiable currents in these cells was examined by voltage–clamp, using KCl (or in some cases KOAc) electrodes, in 17 hippocampal neurons. These included a noninactivating time- and voltage-dependent K current, triggered by depolarizing the cell positive to around -60 mV (I_M) (10), a Ca-activated K current (I_C), a time- and voltage-dependent K conductance activated by raised intracellular Ca (16), and a mixed Na/K current (I_Q) (10). I_M and I_C were studied by holding neurons at a potential of about -30 mV and making 1-sec jumps to both hyperpolarized and depolarized levels. Hyperpolarization resulted in a time-dependent turn-off of I_M to give an inward current relaxation and associated conductance decrease (10); depolarizing steps initiated a further outward current partly due to I_C (16). After more than 30 min in DTX (50–300 nM), neither I_M nor I_C were affected materially. Holding cells close to rest and then hyperpolarizing them turns on I_Q (10), which is responsible for anomalous rectification in the steady-state CVR. This, too, was unaffected by DTX.

DTX Suppresses the I_A in Hippocampal Neurons. The only consistent effect of DTX (50–300 nM) on a membrane current was apparent when employing the protocol that reveals the I_A; this activates rapidly when the cell membrane potential is positive to about -55 mV and, thereafter, inactivates somewhat more slowly, being completely inactivated in the steady-state positive to approximately -60 mV (7). In this study, I_A was initiated by stepping positive to about -55 mV from a much more negative holding potential (Fig. 2A, trace 1) or by returning to a depolarized potential following a hyperpolarizing prepulse beyond -60 mV (Fig. 2A, trace 2) (7). I_A, which activates too rapidly to be resolved by the single electrode clamp and appears as a transient outward current that declines with a time constant of about 50 msec, contributes to an outward rectification in the near-instantaneous CVR (Fig. 2B). Cells held at -45 mV and subjected to hyperpolarizing clamps beyond -60 mV displayed transient outward currents upon return to the holding potential; the current amplitude was dependent on the amplitude of the prepulse, and a plot of this relationship (the inactivation characteristic for the current) is also shown in Fig. 2B. The form of these CVRs is similar to that reported (7) but the currents observed here in CA_1 neurons were about one-fifth the amplitude of those from CA_3 cells. I_A reversed between -70 and -80 mV; its reversal was shifted in a positive direction in high K medium (17, 18). The I_A triggered by jumping from -70 to -40 mV was reduced and then eliminated by increasing the initial concentration of K from 3 to 15 or 25 mM. Contamination of I_A by a fast Ca-activated K current (19) was ruled out by recording the current unscathed in medium containing 200 μM Cd (see below).

As illustrated in Fig. 2A, traces 3 and 4, and B, DTX suppressed I_A whichever way it was triggered. This current is sensitive to 4AP in a variety of mammalian and nonmammalian neurons (6, 17, 18, 20). The effects of both 4AP and DTX on the near-instantaneous CVR for the same hippocampal neuron is shown in Fig. 2C. 4AP (100 μM) applied for 15 min suppressed the transient current, which recovered after a 30 min wash with normal medium (Fig. 2C, *Left*). Application of DTX (290 nM) for 30 min mimicked the action of 4AP (Fig. 2C, *Right*); however, no apparent reversal of these effects was observed on washing the preparation following exposure to toxin at this concentration (but see below). Subtracting the currents generated by depolarizing clamp steps (of varying amplitude) from a negative holding potential in the presence of DTX, from their pretoxin control values, allowed the current decreased by the toxin to be assessed in more detail. As shown in Fig. 2D, DTX blocked
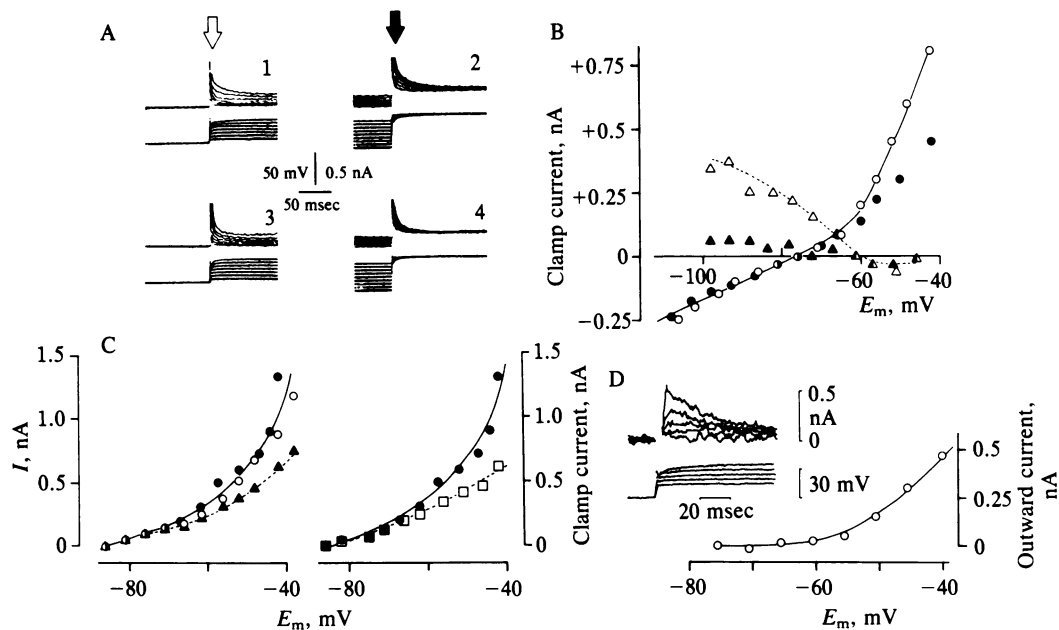
FIG. 2.   Transient outward currents in a hippocampal CA₁ neuron and their inhibition by DTX. (*A*) Oscilloscope records of currents (upper trace of pair) triggered in a hippocampal cell by depolarizing voltage commands (lower trace of pair) from a holding potential of −76 mV (traces 1 and 3) and to a holding potential of −45 mV (traces 2 and 4) before (traces 1 and 2) and 30 min after (traces 3 and 4) exposure to 290 nM DTX. TTX (0.5 μM) was present to eliminate voltage-dependent Na conductances. (*B*) CVRs for the neuron constructed from data similar to that presented in *A*. The near-instantaneous CVR showing the rectification due to the transient current (○) was plotted using values measured about 5 msec after the clamp step (open arrow) in *A* for both depolarizing and hyperpolarizing (not shown) commands. The steady-state inactivation curve (△) was constructed from the current amplitude measured 5 msec after the clamp step (solid arrow) in *A* and the voltage of the hyperpolarizing prepulse. The solid symbols (●, ▲) show the same variables measured in the presence of 290 nM DTX. (*C*) Effects of 4AP and DTX on near-instantaneous CVR. *Left*: control CVR (○) of values measured as in *B* at the instant indicated (open arrow in *A*) but from a holding potential of −86 mV and CVRs redetermined after exposure to 100 μM 4AP (▲) and following a 30-min wash (●). *Right*: the latter CVR (●) served as a control for determination of the CVR after exposing the slice to 290 nM DTX (□) for 30 min. (*D*) Characterization of the DTX-sensitive current. (*Inset*) Superimposed traces of the current eliminated in the above cell by a 30-min exposure to DTX for positive voltage jumps of 15–35 mV from a holding potential of −76 mV. The traces were produced by digitally subtracting the currents observed in the presence of DTX from those initiated by the same voltage jumps before toxin was applied. The peak difference current is plotted against voltage and is the activation curve for the DTX-sensitive current.

specifically a transient outward current with an activation threshold of about −55 mV; furthermore, the decay of this current was kinetically indistinguishable from that of I_A.

Lower doses of DTX were effective in suppressing I_A but with a longer latency to the maximal effect; after 1 hr in 50 nM DTX, I_A was substantially reduced (Fig. 3*A*, traces 1 and 2). Fig. 3*A*, trace 3, shows that the effect of the toxin became more pronounced when the concentration of DTX was increased from 50 to 350 nM, to give in this case almost complete abolition of the I_A. It should be noted, however, that prolonged exposure of the slice to these higher toxin concentrations also caused a decrease in the cell-leak conductance. Fig. 3*B*, traces 1–3, shows another experiment where 50 nM DTX was employed. After 1-hr exposure to toxin the I_A was reduced by 45% (when allowing for leak subtraction) (Fig. 3*B*, trace 2); further washing for 2 hr with control medium induced a partial, but incomplete, recovery (Fig. 3*B*, trace 3), which was manifest in the immediate CVR (Fig. 3*C*) determined from a holding potential of −72 mV. When the cell was held at −53 mV, at which potential I_A is inactivated, no change in other outward currents could be detected by inspection of the steady-state CVR (Fig. 3*C*). To summarize three experiments with exposures to 50 nM DTX for 1 hr, there was a 39% reduction in immediate outward current; following potential steps from values more negative than −65 mV to around −40 mV, this current was reduced from 1628 ± 600 to 987 ± 332 pA, whereas the steady-state current changed by only 42 ± 87 pA. By subtracting the steady-state current from the immediate current to estimate the magnitude of I_A, a reduction in I_A greater than 60% was observed. To avoid protracted experiments, seven cells were

treated with 300 nM DTX for 0.5 hr only; this caused a 76% reduction in I_A: immediate outward current elicited as above declined from 1101 ± 286 to 637 ± 63 pA (significant at $P <$ 0.05, Wilcoxon test) with an average reduction of the corresponding steady-state current of only 39 ± 71 pA (not significant).

Evidence for a Direct Membrane Effect of DTX. Spontaneous release of transmitter still persisted in TTX; to eliminate the possibility that DTX was having its observed action indirectly via enhanced release of this sort, the toxin was applied to four more neurons with 300 μM Cd present. I_A was not changed materially by Cd (cf. ref. 7), and 300 nM DTX suppressed this current after a 20- to 30-min exposure; in Cd-containing media the immediate outward current elicited as before was reduced to 591 ± 68 pA from a pretoxin value of 849 ± 81 pA. The unclamped resting potential was not affected by DTX treatment in the presence of Cd (median membrane potential change, 1 mV hyperpolarization). In addition to tests with DTX, the more toxic homologue toxin I was administered in the presence of Cd, and it gave almost complete block of I_A after a 30 min exposure at 150 nM (Fig. 4); a substantial reduction of I_A (around 40%) was observed after 30 min in only 10 nM toxin I.

Detection of a Specific Acceptor Protein for ^{125}I-DTX on Synaptosomal Membranes. The ^{125}I-DTX prepared was of high specific radioactivity (≈250 Ci/mmol; 1 Ci = 37 GBq); its toxicity on injection into rat brain was very similar to that of DTX, and its acceptor affinity (see below) was also unaltered (2, 8). Saturable binding of ^{125}I-DTX to the membrane fraction of cerebrocortical synaptosomes was observed, with nonsaturable labeling representing a low pro-
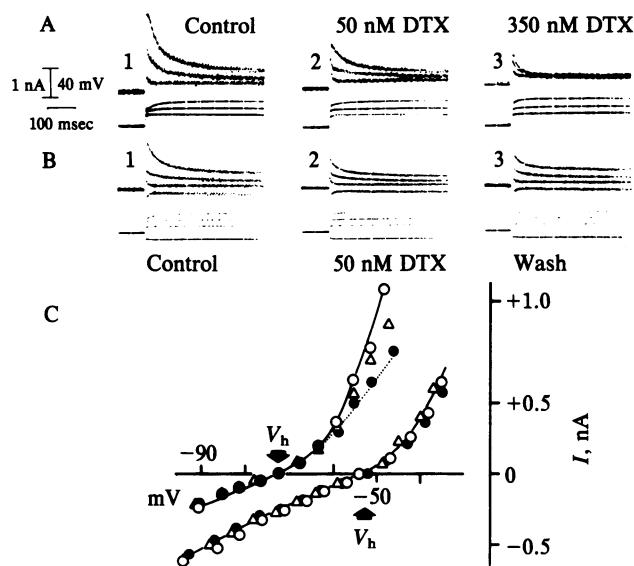
FIG. 5. Autoradiographic localization of saturable acceptor sites for ^{125}I-DTX in rat brain. Coronal sections of whole prefixed brain were dry mounted on slides and incubated with 2.5 nM ^{125}I-DTX in Krebs buffer containing serum albumin (1 mg/ml) at 22°C for 30 min. After rapid washing at 4°C, the sections were dried and exposed to Ultrofilm for 2 days. Following development, photographs were printed directly from the film (×16). Labeled areas are identified as follows: p, pyramidal cells; g, granule cells; m, molecular layer; sr, stratum radiatum; so, stratum oriens; cc, corpus callosum. Note that the electrophysiological recordings were made in the dorsal (upper) part of the pyramidal cell layer (p).

FIG. 3. Concentration dependence of DTX effects on I_A in hippocampal neurons. (*A*) Transmembrane currents (upper trace of pair) driven by voltage steps from −75 mV shown (lower traces) before (control) and 1 hr after exposure of the brain slice to 50 nM DTX. An additional 300 nM DTX was then added and trace 3 was taken 20 min later. In this example and that of *B*, TTX (0.5 μM) was present. (*B*) Currents depicted as in *A* and recorded from another neuron (trace 1) before, (trace 2) after 1 hr in 50 nM DTX, and (trace 3) following 2 hr wash. The indicated voltage jumps were made from a holding potential (V_h) of −72 mV. (*C*) CVRs plotted for the cell shown in *B* before (○), after 1 hr in 50 nM DTX (●) and following 2 hr wash (△). Two families of curves are depicted: near-instantaneous CVRs determined from a V_h of −72 mV by measuring currents 2 msec after the clamp step was initiated; steady-state CVRs, measured at the end of 1 sec clamp steps from a V_h of −53 mV at which potential I_A is inactivated. The ordinate zero refers to the steady current required to hold the cell at V_h (i.e., the 237 pA difference between holding currents for V_h = −53 mV and −72 mV has been ignored).

portion (<15% at 1.5 nM) of the total. From Scatchard analysis of the data, a noninteracting population of sites (B_{max} = 1.1 pmol/mg of protein) with high affinity (K_D = 0.3 nM) was apparent (8). Binding to acceptor sites in synaptosomal membranes from hippocampus showed similar properties (A. L. Breeze and J.O.D., unpublished results). The proteinaceous nature of the binding component was demonstrated by its sensitivity to proteolytic enzymes and heat treatment (2, 5). Specificity of the acceptor for DTX and its homologues was ascertained from competition studies; the affinity of toxin I (K_I = 0.06 nM), a congener of DTX, was about 8-fold higher than DTX. It is noteworthy that relative
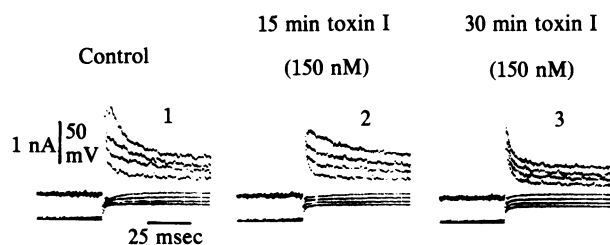
to the latter the toxicity of toxin I is also higher (≈5-fold), on intracerebroventricular injection into rats (5). In contrast, inhibitory presynaptically active proteins (e.g., taipoxin, crotoxin, or apian phospholipase A$_2$) were ineffective when their enzymatic activities were suppressed in Ca-free medium (2). The extent of inhibition of DTX binding by β-bungarotoxin was minimal when equal nanomolar concentrations of both toxins were used; at high concentrations (>1 μM) of β-bungarotoxin, antagonism was observed but even then the inhibition was partial, either with or without Ca^{2+} (2).

Quantitation and Autoradiographic Localization of ^{125}I-DTX Acceptor in Brain Sections. The approximate values of K_D and B_{max} (about 1.0×10^{-9} M; 0.8–1.1 pmol of ^{125}I-DTX/mg of protein) obtained for ^{125}I-DTX bound to sections of whole brain were not dissimilar to those measured more precisely on synaptosomes. Light-microscope autoradiograms of whole brain sections labeled with ^{125}I-DTX demonstrated extensive deposition of silver grains, located in very discrete regions of hippocampus (Fig. 5) and cerebellum (data not shown). In contrast, control samples treated with ^{125}I-DTX in the presence of a 100-fold excess of DTX gave virtually no labeling. Examination of these samples, after visualizing the different cell layers by staining with pyronin Y, showed that a high content of silver grains was present in terminal-rich areas (strata oriens and radiatum, molecular layer of dentate gyrus) in hippocampus (Fig. 5) or cerebellum. Although fewer grains were associated with regions containing predominantly cell bodies (e.g., pyramidal and granule cell layers), areas rich in white matter (e.g., corpus callosum and habenular nucleus) showed appreciable labeling; other regions (e.g., cerebral cortex) had a fairly uniform distribution of grains (Fig. 5).

FIG. 4. Effects of toxin I on I_A. (*A*) Transmembrane currents driven (upper trace in pair) by stepping the membrane potential from a V_h of −70 mV (lower traces) and recorded from a hippocampal cell bathed in control medium containing 0.5 μM TTX, 2 mM Cs (to block I_Q) (10) and 200 μM Cd (to block Ca currents). The action of Cd did not materially affect the currents over the voltage range indicated. Responses (trace 1) before and (trace 2) 15 or (trace 3) 30 min after the addition of 150 nM toxin I are shown.
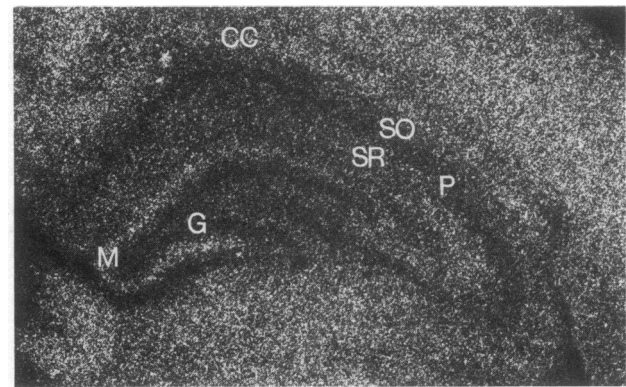
DISCUSSION

The results presented here show that in the soma-dendritic membrane of hippocampal CA$_1$ neurons, DTX specifically suppresses a transient, voltage-sensitive, outward current identified in hippocampal CA$_3$ cells as I_A (7). This current serves to limit the excitability of neurons at resting potentials negative enough for I_A inactivation to be removed (18); the transient current initiated by depolarization exerts a short-lived hyperpolarizing influence and reduces excitability both by means of this and also the conductance increase associ-

ated with channel opening. Thus, attenuation of I_A by DTX could account for the observed facilitation of Ca spikes and enhancement of cell excitability because hippocampal neurons possess a complementary fast Ca current that operates in the same voltage range as I_A (13) (Fig. 1). Moreover, DTX does not directly affect either of the Ca currents present in these cells (13, 14) or other identified currents: I_M, I_C, and I_Q (10, 16).

DTX mimics the action of 4AP on I_A in hippocampal neurons; it is up to 2000 times more effective in molar terms. Also, enhancement of synaptic transmission and convulsive activity has been seen in the hippocampal preparation with DTX (2, 3) or 4AP (21). Such synaptic facilitation could arise from a suppression of I_A at the nerve terminal and, indeed, evidence for this exists in the case of 4AP (22); in addition, this potentiation by DTX or 4AP would be compounded by an increase in cell-body excitability also resulting from inhibition of I_A. Identification of acceptor sites at axonal, terminal, and soma-dendritic sites by light and electron-microscopic autoradiography (unpublished data) is consistent with these effects of DTX. Further support for the involvement of such acceptors in the lethal-convulsive action is provided by the proportionality in the central neurotoxicities of DTX or 4 homologues (5) to their binding affinities for synaptic membranes. In agreement, the effectiveness of DTX and toxin I in reducing I_A is in proportion to their neurotoxicities and binding affinities. However, the nature of this involvement of the acceptors in the inhibition of I_A by DTX remains unclear. For example, the minimum effective dose of DTX was much greater than its K_D but approximated to the calculated concentration required in the ventricular fluid to cause death. Restricted access of this very basic and relatively large molecule to the recording locus (200–300 μm from the surface of the slice) may account for this discrepancy and for the slow onset of action at low DTX concentrations, together with the lack of reversibility seen at higher doses. Using only nanomolar amounts of toxin, the change in DTX concentration within the depth of slice would be greatly delayed by the large acceptor content, since the value of 1 pmol of toxin/mg of protein found for tissue sections is equivalent to micromolar concentration in the membrane area bathed by the extracellular fluid (assuming 5–10% extracellular space). Indeed, little penetration of ^{125}I-DTX to the center of brain slices was noted by light-microscope autoradiography using toxin concentrations, section thickness, and incubation times similar to those employed for electrophysiological measurements (unpublished observations).

The aminopyridines and DTX have a similar profile of action in the periphery; they enhance neuromuscular transmission (1), inhibit I_A in rat sympathetic ganglia (ref. 17; A. A. Selyenko and J.V.H., unpublished results), and suppress a K conductance in nodose ganglia (C. Stansfeld, S. Marsh, J.V.H., and D. Brown, unpublished results), which more resembles I_A than the delayed rectifier (23). Actions of 4AP appear to be more widespread than those of DTX since in a range of axonal membranes they include blockade of the delayed rectifier current (24–27) and prolongation of the action potential. Such an action of 4AP on axonal membrane seems also to occur centrally (28, 29) whereas DTX does not markedly prolong the duration of extracellularly recorded action potentials from presynaptic fibers in the hippocampus (2, 3). Neither 4AP nor DTX appear to broaden somatically recorded action potentials (refs. 18 and 21; J.V.H., unpublished observations) at concentrations which substantially reduce I_A and increase cell excitability. These observations lead us to conclude that DTX is a specific probe for an acceptor site that could directly or indirectly reduce this transient K conductance. Such inhibition of I_A by the toxin is sufficient to explain its convulsant action in terms of

enhanced cell excitability (see above) and facilitated synaptic transmission. Although an abundance of the synaptic acceptors implies a predominant effect of DTX at the terminals, the importance of this relative to its observed action on the cell body, or perhaps axonal membranes, cannot be quantified readily; additionally, the differential sensitivity to β-bungarotoxin of a minority of the acceptors emphasizes some heterogeneity (2). Furthermore, variants of the I_A channel/macromolecular complex exist, at least in the few species examined because DTX does not affect I_A in neurons of the buccal ganglion of *Aplysia* (L. Tauc, G. Baux, M. Boulain, and J.O.D., unpublished data) or in sea-urchin eggs (J.V.H., unpublished observations). Progress being made with the purification of these acceptors, following their solubilization in biologically active form (8), should allow these important questions to be addressed.

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