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α-Latrotoxin of black widow spider venom depolarizes the plasma membrane, induces massive calcium influx, and stimulates transmitter release in guinea pig brain synaptosomes

(plasma membrane potential/mitochondrial membrane potential/calcium transport/γ-aminobutyrate/noradrenaline)

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ABSTRACT **The effect of α-latrotoxin from black widow spider venom upon guinea pig cerebral cortical synaptosomes is described. Plasma membrane potential ($\Delta\psi_p$), *in situ* mitochondrial membrane potential ($\Delta\psi_m$), Ca^{2+} transport, γ-amino[^{3}H]butyrate release, [^{3}H]noradrenaline release, and synaptosomal ATP were monitored under parallel conditions. Potentials were determined both isotopically and with a tetraphenylphosphonium-selective electrode. α-Latrotoxin depolarizes $\Delta\psi_p$ selectively, both in the presence and absence of Ca^{2+}. A slight toxin-induced depolarization of $\Delta\psi_m$ is a consequence of a massive Ca^{2+} uptake across the plasma membrane. Depolarization of $\Delta\psi_p$ is insensitive to tetrodotoxin, and Ca^{2+} entry is only partially inhibited by verapamil. Release of [^{3}H]noradrenaline and γ-amino[^{3}H]butyrate is markedly stimulated by the toxin in the presence of Ca^{2+}, and this effect is only slightly reduced in Ca^{2+}-free conditions.**

The component of the venom of the black widow spider (*Latrodectus mactans tredecimguttatus*) that is toxic towards vertebrate neurons (for review, see ref. 1) is a protein of M_r 130,000 named α-latrotoxin (2–10). At the neuromuscular junction, the toxin causes massive release of acetylcholine and depletion of synaptic vesicles (1–4, 11, 12); moreover, it releases acetylcholine, noradrenaline, dopamine, and γ-aminobutyrate from cerebral cortical slices (5–7), synaptosomes (8, 10, 13), and one neurosecretory cell line (9). A small number of high-affinity binding sites for the toxin are located specifically on the presynaptic plasma membrane (6, 10), and occupancy of these correlates well with the subsequent release of neurotransmitter (10). In addition, the toxin is able to create stable conductance channels in protein-free black lipid membranes (14).

A number of aspects of α-latrotoxin action are unclear. In particular, there is no consensus as to the role of Ca^{2+}. Whereas there is agreement that the release of acetylcholine and γ-aminobutyrate is independent of external Ca^{2+} (3, 4, 7, 12, 13), the Ca^{2+} dependency of catecholamine release appears dependent upon the experimental conditions and target membrane (5, 7–9). In addition, there is a contradiction between the results obtained with neurosecretory PC12 cells, in which a primary effect of the toxin appears to be a massive uptake of Ca^{2+} across the plasma membrane (9), and those obtained with rat cerebral cortical synaptosomes, in which no effect on Ca^{2+} flux could be detected (13).

The possibility of α-latrotoxin-induced massive Ca^{2+} uptake across the presynaptic membrane raises a number of unanswered questions about the effects of the toxin on the energy levels in the cell. In particular, it is unclear whether the intraterminal mitochondria would be able to cope with this Ca^{2+} influx. Indeed swollen and disorganized mitochondria can be observed in electron micrographs of presynaptic expansions both at neuromuscular junctions (1–4, 11) and in cortical slices (7) treated with α-latrotoxin in the presence of external Ca^{2+}. De-energization of the mitochondria resulting in a collapse of the mitochondrial membrane potential ($\Delta\psi_m$) would not only prevent mitochondrial synthesis of ATP but also would destroy the ability of the mitochondria to regulate the upper limit of the cytosolic free Ca^{2+} concentration (for reviews, see refs. 15–17), with a resultant risk of cell death (18).

Recent developments in the study of isolated synaptosomes have enabled Ca^{2+} transport, $\Delta\psi_m$, and the plasma membrane potential ($\Delta\psi_p$) to be determined simultaneously (15, 18–21). In this paper we use these techniques and introduce the application of the tetraphenylphosphonium (Ph_4P^+)-selective electrode (22) for the monitoring of synaptosomal potentials in suspension.

EXPERIMENTAL PROCEDURES

Synaptosomes. Synaptosomes were prepared from the cerebral cortices (including corpus striatum) of Duncan–Hartley strain guinea pigs age 4–8 wk as described (19–22). The synaptosomes were stored as a pellet in 250 mM sucrose/5 mM 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethane sulfonate (Tes; Na^+ salt), pH 7.0, at 0°C for not more than 4 hr before use. Protein was determined by the biuret method (23).

Ph_4P^+-Selective Electrode. A polyvinyl chloride membrane selectively permeable to Ph_4P^+ was formed essentially as described by Kamo *et al.* (24), by allowing a solution containing 0.34 mg of tetraphenylboron (Ph_4B^-; Na^+ salt), 16 mg of polyvinyl chloride (high molecular weight), 57 μl of dioctylphthalate, and tetrahydrofuran (to a final volume of 500 μl) to evaporate on a glass plate constrained by a glass cylinder of 1.9-cm diameter. The resulting membrane was then glued to the place of an exhausted conventional membrane of a radiometer type 2112a Ca^{2+}-selective electrode. The internal filling solution was 10 mM Ph_4P^+/10 mM NaCl. The electrode was assembled into a Perspex chamber of 1.7-ml capacity containing a bridge to a KCl reference electrode.

Incubation Media. In all experiments the synaptosomal pellet was resuspended in 122 mM NaCl/3.1 mM KCl/0.4 mM KH_2PO_4/5 mM $NaHCO_3$/20 mM Tes (Na^+ salt)/10 mM D-glucose/1.2 mM $MgSO_4$/16 μM bovine serum albumin (fraction V), pH 7.4, at 30°C. Further initial additions are specified

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Abbreviations: FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; Ph_4B^-, tetraphenylboron; Ph_4P^+, tetraphenylphosphonium; Ph_3MeP^+, triphenylmethylphosphonium; $\Delta\psi_p$, plasma membrane potential; $\Delta\psi_m$, mitochondrial membrane potential; Tes, 2-{[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]amino}ethane sulfonate.

below. The synaptosomal suspension (1.5 mg of protein per ml of incubation mixture) was preincubated for 15 min in a shaking water bath prior to initiation of the experiment.

Electrode Measurements of Ph_4P^+ Accumulation. Preincubated suspension (1.7 ml) was transferred to the Ph_4P^+-electrode chamber; 1 μM Ph_4P^+ was added together with 1.3 mM $CaCl_2$ or 1 mM EGTA as appropriate. The uptake of Ph_4P^+ by the synaptosomes was recorded continuously, and the accumulation of Ph_4P^+ (expressed as the ratio of the overall concentration of the cation within the synaptosome, ignoring compartmentation, to the residual concentration in the medium, $Ph_4P^+_{in}/Ph_4P^+_{out}$) was calculated by assuming a Nernstian response for the electrode over the range 0.2–1 μM. The synaptosomal volume was 3.23 μl/mg of protein (19).

Isotopic Measurements of $\Delta\psi_p$ and $\Delta\psi_m$. The distribution of $^{86}Rb^+$ and tritium-labeled triphenylmethylphosphonium ion ($[^3H]Ph_3MeP^+$) between synaptosomes and medium was used to estimate $\Delta\psi_p$ and $\Delta\psi_m$ as described (19), but synaptosomes were separated from the incubation medium by centrifugation through silicone oil (Corning 550)/dinonylphthalate 1:1 (vol/vol) (20–22). No correction was made for nonideal activity coefficients for Ph_3MeP^+ in the cytosolic or matrix compartments. The initial preincubation was in 0.2 μM $[^3H]Ph_3MeP^+$ (0.2 μCi/ml; 1 Ci = 3.7×10^{10} becquerels)/50 μM ^{86}RbCl (0.1 μCi/ml)/0.5 μM [^{14}C]sucrose (0.2 μCi/ml)/3 μM tetraphenylboron (Ph_4B^-; Na^+ salt). Synaptosomal pellets were extracted for counting as described (20–22).

Ca^{2+} Transport. The uptake of ^{45}Ca and $\Delta\psi_p$ were determined simultaneously as described (21). The initial additions to the preincubation were [^{3}H]sucrose (1.4 μCi/ml) and ^{86}RbCl (0.14 μCi/ml). $^{45}CaCl_2$ (1.3 mM; 0.6 μCi/ml) was added at the end of the preincubation period. Immediately prior to centrifugation, the aliquots were mixed with 2.5 mM EGTA/5 μM ruthenium red (final concentrations) to remove superficial Ca^{2+} (20).

γ-Amino[^{3}H]butyrate and [^{3}H]Noradrenaline Content. The initial additions to the preincubation were 0.5 μM [^{14}C] sucrose (0.2 μCi/ml), 50 μM ^{86}RbCl (0.1 μC/ml), and either 0.5 μM γ-amino[^{3}H]butyrate (0.42 μCi/ml) plus 10 μM aminoxyacetate or 0.25 μM [^{3}H]noradrenaline (0.3 μCi/ml) plus 5 μM nialamide. In the noradrenaline experiment, 50 μM imipramine was added 1 min prior to addition of α-latrotoxin. Aliquots were centrifuged through silicone oil.

Other Assays. ATP was determined in perchloric acid extracts of total synaptosomal incubations by the luciferin/luciferase technique (25).

Materials. α-Latrotoxin was purified from the venom glands of female European black widow spiders (2) and stored at −80°C in 50 mM Tris·HCl, pH 8.2. $[^3H]Ph_3MeP^+$ was obtained from New England Nuclear (Dreieich, Federal Republic of Germany). All other radioisotopes were obtained from the Radiochemical Centre (Amersham, Bucks, United Kingdom). Ph_4P^+ was obtained from Fluorochem (Glossop, United Kingdom). Ph_4B^+, oligomycin, ouabain, veratridine, tetrodotoxin, and most other reagents were obtained from Sigma (Poole, Dorset, United Kingdom), and verapamil (Cordilox) was obtained from Abbott Laboratories (Queenborough, Kent, United Kingdom).

RESULTS

Response of the Ph_4P^+-Selective Electrode. Fig. 1 shows the response to the specific depolarization of the plasma membrane induced by high K^+ concentration or ouabain and the response to the specific depolarization of the *in situ* mitochondrial inner membranes induced by rotenone/oligomycin or by the proton translocator carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (19). The overall accumulation of the cation was

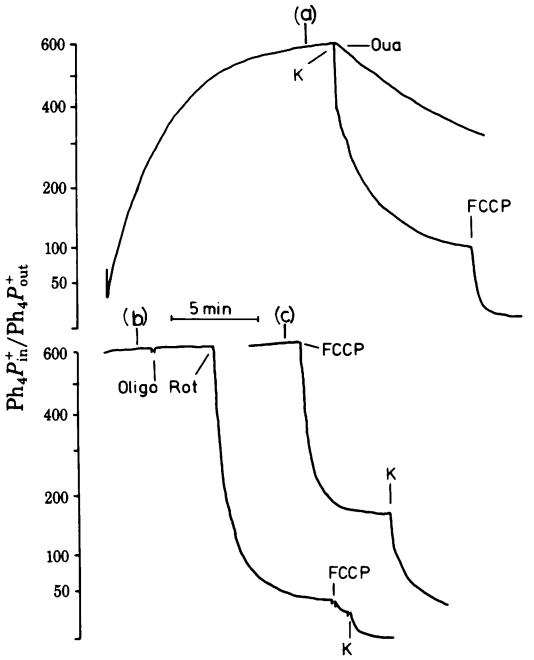

FIG. 1. Response of the Ph_4P^+-selective electrode to changes in $\Delta\psi_p$ and $\Delta\psi_m$. Ph_4P^+ (1 μM) was added to synaptosomal incubation in the presence of 1.3 mM $CaCl_2$. Where indicated, the following additions were made: 35 mM KCl (K), 0.3 mM ouabain (Oua), 2 μM FCCP, 2 μg of oligomycin per ml (Oligo), and 2 μM rotenone (Rot). The initial time-course of Ph_4P^+ uptake is shown for trace a.

the consequence of uptake across both the plasma membrane and the *in situ* mitochondrial inner membrane. The 90% decrease after addition of rotenone in the presence of oligomycin (Fig. 1, trace b) suggests that about 90% of the Ph_4P^+ accumulated within the terminal is further transported into the mitochondrial matrices. The decrease after addition of FCCP (Fig. 1, trace c) was somewhat less marked because of the use of a suboptimal concentration of the proton translocator. Thus, the electrode responds rapidly to changes in either potential, and potential-independent binding appears minimal.

The addition of 3 nM α-latrotoxin to polarized synaptosomes induced an extensive efflux of Ph_4P^+ after a lag of ≈30 sec (Fig. 2, trace a). Comparison of the extent of additional efflux induced by high K^+ concentration with that under control conditions (Fig. 1, trace a) suggests that the major effect is upon $\Delta\psi_p$. The insensitivity of the toxin-induced transmitter release to tetrodotoxin is well established (7, 13), and a 100-fold molar excess of tetrodotoxin was without effect upon the subsequent extent or rate of Ph_4P^+ efflux (Fig. 2, trace b). Addition of α-latrotoxin to synaptosomes that had incubated for 14 min in Ca^{2+}-free medium in the presence of EGTA—conditions which are known to deplete the synaptosomal Ca^{2+} (20)—still induced a Ph_4P^+ efflux, although the rate and extent were somewhat diminished [compare the effect of 3 nM toxin in the absence of Ca^{2+} (Fig. 2, trace c) with that of 1 nM toxin in the presence of Ca^{2+} (Fig. 2, trace f)]. The extent of the residual efflux induced by FCCP addition suggests that a toxin-dependent decrease in $\Delta\psi_m$ also occurs, but that this is less marked in Ca^{2+}-free media.

The concentration dependency of the α-latrotoxin-induced depolarization (Fig. 2, traces d–h) showed that as little as 0.3 nM toxin caused a significant depolarization and that the effect was almost maximal at 3 nM. This concentration range correlates rather well with the observed K_d for the toxin-binding sites on the rat or dog synaptosomal plasma membrane (6, 10).

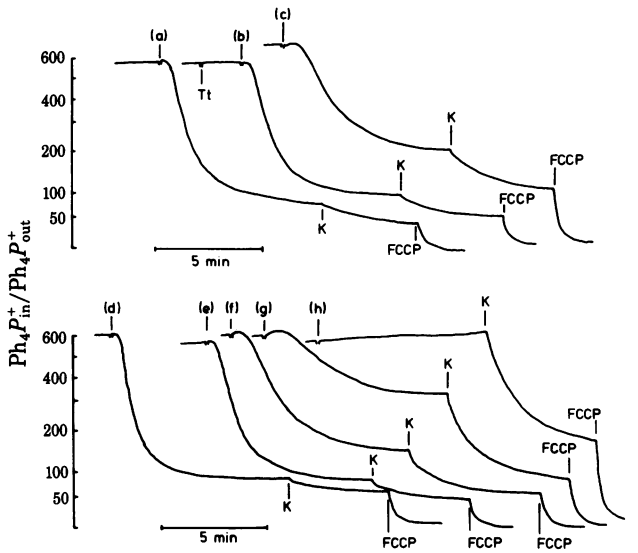

FIG. 2. Effect of α-latrotoxin on the accumulation of Ph_4P^+. After a 15-min preincubation, 1 μM Ph_4P^+ was added to synaptosomal incubation together with 1.3 mM $CaCl_2$ (traces, a, b, d–h) or together with 1 mM EGTA (trace c); 35 nM KCl (K) and 10 μM FCCP were added where indicated. (*Upper*) Addition of 3 nM α-latrotoxin to Ca^{2+}-containing incubations (trace a) preceded by 0.3 μM tetrodotoxin (Tt) (trace b) or to the Ca^{2+}-free EGTA incubation (trace c). (*Lower*) Depolarization was initiated by the following concentrations of α-latrotoxin: 10 nM (trace d), 3 nM (trace e), 1 nM (trace f), and 0.3 nM (trace g). Trace h is the control.

The Effect of α-Latrotoxin on $\Delta\psi_p$ and $\Delta\psi_m$. The Ph_4P^+-selective electrode did not allow $\Delta\psi_p$ and $\Delta\psi_m$ to be calculated directly. In order to obtain more precise information (within the constraints of indeterminate activity coefficients), the dual-isotope technique was used with $^{86}Rb^+$ and $[^3H]Ph_3MeP^+$ (19). Fig. 3 shows the relative effects of α-latrotoxin on $\Delta\psi_p$ and $\Delta\psi_m$ at two toxin concentrations. In the absence of Ca^{2+} no effect on $\Delta\psi_m$ could be detected, whereas, even in the presence of Ca^{2+} and with a high concentration of toxin, $\Delta\psi_m$ only decreased by about 10 mV. These results confirm, therefore, that the toxin

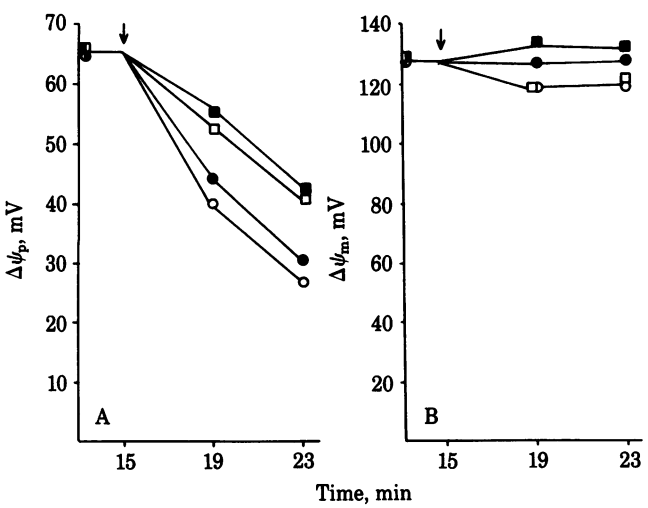

FIG. 3. Effect of α-latrotoxin on $\Delta\psi_p$ and $\Delta\psi_m$. Synaptosomes were incubated in the presence of $^{86}Rb^+$, $[^{14}C]$sucrose, and $[^3H]Ph_3MeP^+$ with the addition at $t = 0$ of 1.3 mM $CaCl_2$ (○, □) or 1 mM EGTA (●, ■). After a further 15 min, α-latrotoxin was added (arrow) to a final concentration of 1 nM (□, ■) or 3 nM (○, ●). At defined times, aliquots were centrifuged through silicone oil for the simultaneous determination of $\Delta\psi_p$ (*A*) or $\Delta\psi_m$ (*B*).

Table 1. The effects of α-latrotoxin on $\Delta\psi_p$

Addition at $t = 0$	$\Delta\psi_p$, mV: Before α-latrotoxin addition	$\Delta\psi_p$, mV: After α-latrotoxin addition
Control	64	27
KCl, 35 mM	26	7
Veratridine, 100 μM + ouabain, 0.3 mM	34	13
Veratridine, 100 μM + tetrodotoxin, 0.3 μM	66	25
FCCP, 10 μM + oligomycin, 2 μg/ml	57	18

Conditions were exactly as described in the legend to Fig. 3. Agents were added at $t = 6$ min together with 1.3 mM $CaCl_2$ after a 15-min preincubation. Duplicate samples were taken after a further 14 min (1 min prior to the addition of α-latrotoxin) and also after 23 min (8 min after toxin).

acts selectively at the plasma membrane and that the mitochondria remain bioenergetically competent under the conditions used in this study.

To further substantiate this conclusion, total synaptosomal ATP was determined. In the presence of ouabain (to prevent a decrease in ATP because of an enhanced Na^+-cycling at the plasma membrane) and in the absence of Ca^{2+}, we found that an 8-min exposure to 3 nM α-latrotoxin lowered total synaptosomal ATP by only 37%. When the toxin was applied in the Ca^{2+}-containing medium, depletion of ATP was more marked (−70%).

Table 1 shows that (*i*) α-latrotoxin was able to enhance the depolarization of the plasma membrane induced by 35 mM KCl or by ouabain/veratridine; (*ii*) the tetrodotoxin concentrations used in this study were adequate to inhibit a veratridine-induced depolarization, confirming that tetrodotoxin is completely without effect on α-latrotoxin-induced depolarization; and (*iii*) α-latrotoxin was effective, even when $\Delta\psi_m$ was selectively abolished by the combination of FCCP and oligomycin (19).

Ca^{2+} Fluxes Associated with α-Latrotoxin Action. The addition of 0.3 nM α-latrotoxin to synaptosomes incubated in Ca^{2+}-containing media resulted in an extensive and rapid up-

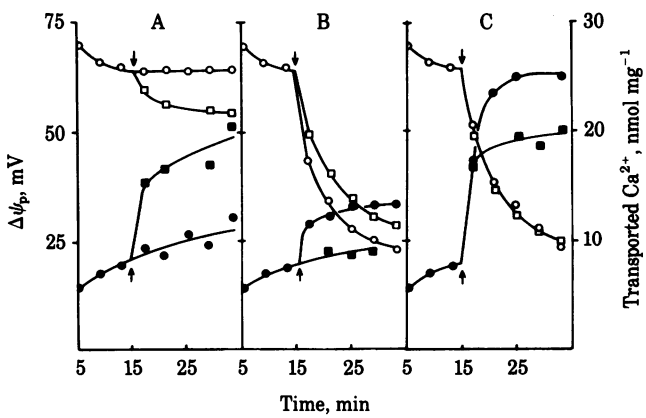

FIG. 4. The effects of α-latrotoxin and high K^+ concentration on $\Delta\psi_p$ (○, □) and Ca^{2+} transport (●, ■) across the synaptosomal plasma membrane. Synaptosomes were preincubated in the presence of ^{86}Rb and $[^3H]$sucrose for 15 min prior to the addition of 1.3 mM $CaCl_2$ at $t = 0$. After a further 15 min incubation, the following additions were made (arrow). (*A*) 0.3 nM α-latrotoxin (□, ■). (*B*) 35 mM KCl (○, ●) and 35 mM KCl/100 μM verapamil (□, ■). (*C*) 3 nM α-latrotoxin (○, ●) and 3 nM α-latrotoxin/100 μM verapamil (□, ■). Control (○, ●) is in *A*.

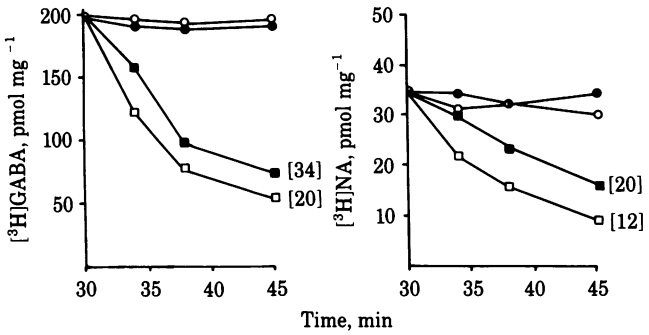

FIG. 5. The Ca^{2+}-dependency of the effects of α-latrotoxin on the retention of γ-amino[^{3}H]butyrate ([^{3}H]GABA) and [^{3}H]noradrenaline ([^{3}H]NA) by synaptosomes. Synaptosomes were preincubated as described. ^{86}Rb and [^{14}C]sucrose were present at $t = 0$. At $t = 15$ min, 1.3 mM $CaCl_2$ was added (○, □) or omitted (●, ■). After a further 15 min, 3 nM α-latrotoxin was added (□, ■). Controls were performed in the absence of toxin (○, ●). Numbers in parentheses are the $\Delta\psi_p$ in mV at the termination of each experiment.

take of Ca^{2+} into the synaptosome (Fig. 4*A*). The uptake of Ca^{2+} was considerably greater than that occurring through the voltage-sensitive Ca^{2+} channels in response to a depolarization of $\Delta\psi_p$ induced by high K^+ concentration (22), even though K^+ decreased $\Delta\psi_p$ to a much greater extent than did 0.3 nM toxin (Fig. 4*B*). When 3 nM α-latrotoxin was used to decrease $\Delta\psi_p$ to an extent comparable to 35 mM KCl (Fig. 4*C*), an even more extensive uptake of Ca^{2+} was observed. In contrast to the complete inhibition by verapamil of the K^+-induced Ca^{2+} uptake (Fig. 4*B*), there was only a partial inhibition of the toxin-induced uptake (Fig. 4*C*). This suggests that the α-latrotoxin-induced Ca^{2+} uptake has two components. The smaller component is verapamil-sensitive and occurs through the voltage-sensitive Ca^{2+} channel as a secondary consequence of the toxin-induced depolarization, whereas the large verapamil-insensitive uptake is induced by the toxin directly.

Ca^{2+}-Dependency of the Toxin-Induced Release of Tritiated Neurotransmitters. The addition of 3 nM α-latrotoxin to synaptosomes that had accumulated γ-amino[^{3}H]butyrate or [^{3}H]noradrenaline resulted in an extensive release of the transmitters (Fig. 5). The release of both transmitters was significantly greater in the presence than in the absence of Ca^{2+}. The lower efficacy of α-latrotoxin on transmitter release in Ca^{2+}-free conditions could be correlated with its lower effects on $\Delta\psi_p$ (Fig. 5, values in parentheses). Thus, there is no evidence for a requirement of extracellular Ca^{2+} for the release of either radiolabeled transmitter.

DISCUSSION

Use of the Ph_4P^+-Selective Electrode. There have been a number of recent studies of membrane potentials in synaptosomes (15, 19, 21, 26–29) or neuroblastoma cells (30, 31) utilizing radioisotopes of the lipophilic cations Ph_4P^+ (26, 30) or Ph_3MeP^+ (15, 19, 21, 27–29, 31). There is some controversy as to the extent to which the cations indicate the existence of a significant potential across the inner membranes of the *in situ* mitochondria. We have observed that it is necessary to use low (1 μM) concentrations of cation in order to avoid depolarization of the mitochondria, whereas in the case of Ph_3MeP^+ it is necessary to include Ph_4B^- in order to allow full equilibration across the mitochondrial membrane (19). Thus, the failure of some synaptosomal studies to detect $\Delta\psi_m$ can be ascribed to the use of an excessive concentration of cation (27, 29) or to omission of Ph_4B^- (28). An excessive Ph_3MeP^+ concentration also was used in one neuroblastoma study (31), although the low accumulation found with moderate concentrations of Ph_4P^+ in neuroblastoma–glioma cells (30) suggests that these cells may have difficulty in fully equilibrating these cations.

The present electrode study is entirely consistent with the isotopic investigations of Ph_4P^+ accumulation into synaptosomes by Ramos *et al.* (26) and qualitatively with our previous use of [^{3}H]Ph_3MeP^+ (15, 19, 21). With respect to the latter studies, however, we observe now a higher cation accumulation, which presumably reflects a lower activity coefficient of the more lipophilic Ph_4P^+ in the internal compartments. Our present results illustrate that the Ph_4P^+-selective electrode has considerable possibilities for monitoring qualitative potential changes in cellular suspensions, as long as controls are performed to localize any effect to either the plasma or mitochondrial membrane.

α-Latrotoxin and Membrane Potentials. The observation by Finkelstein *et al.* (14) that α-latrotoxin induced Na^+ and Ca^{2+} conductances in black lipid membranes led them to speculate that the toxin acted by depolarizing the presynaptic membrane and by allowing Ca^{2+} entry. Although there has been one qualitative report that a decrease in the fluorescence quenching of the cationic indicator 3,3′-dipentyl-2,2′-oxacarbocyanine is caused by the crude venom (13), indicative of a membrane depolarization (32), the technique does not permit localization of the effect to an individual membrane, and no quantitation was possible.

The present study shows that α-latrotoxin specifically depolarizes the plasma membrane over a range of toxin concentrations that correlates well with the binding affinity of the toxin in other systems (6, 10) and in guinea-pig synaptosomes (unpublished observations). In the present work, no measurements of Na^+ transport were performed. Thus, no definite information about the mechanism(s) underlying the plasma membrane depolarization (possibly increase in Na^+ conductance) is available as yet. In common with a number of previous studies (8, 13), no effect of tetrodotoxin on the subsequent α-latrotoxin-induced depolarization could be seen. This confirms that α-latrotoxin acts at a site independent from the voltage-sensitive Na^+ channel.

The toxin is highly specific for the plasma membrane because the only effect on the mitochondria is a slight depolarization in the presence of external Ca^{2+}, which can be ascribed to a massive accumulation of Ca^{2+} by these organelles consistent with their swollen appearance under the electron microscope (refs. 3, 4, and 7; unpublished data). However, the maintained $\Delta\psi_m$ indicates that the free cytosolic Ca^{2+} concentration does not rise significantly above 1 μM during toxin-induced Ca^{2+} uptake (due to the further sequestration of the cation by the mitochondria) because any greater increase in cytosolic free Ca^{2+} would inevitably cause a mitochondrial depolarization (for review, see refs. 15–17). Therefore, transmitter release cannot be ascribed to "cell death" (18) consequent upon a Ca^{2+}-induced destruction of the mitochondria.

α-Latrotoxin and Ca^{2+} Transport. In agreement with the effect on PC12 cells (9), binding of the toxin to the plasma membrane induces a massive uptake of Ca^{2+}, which is only partially verapamil sensitive. The extent of such an uptake also suggests that a pathway other than the verapamil-sensitive Ca^{2+} channel is involved. We are unable to explain the failure of Baba and Cooper (13) to detect significant Ca^{2+} fluxes induced by the addition of crude venom to rat synaptosomes.

α-Latrotoxin and Transmitter Release. The marked effect of α-latrotoxin on noradrenaline and γ-aminobutyrate release is only moderately decreased when Ca^{2+} is omitted from the incubation fluid. Whether this reflects a true dependency of

the release process on extracellular Ca^{2+} is doubtful, however, because both the binding of the toxin (ref. 6; unpublished observations) and the toxin-induced depolarization are also partially inhibited in the absence of Ca^{2+}. With regards to the mechanisms of the toxin-induced transmitter release, extensive work demonstrates that, at the neuromuscular junction, acetylcholine is released by massive stimulation of exocytosis both in Ca^{2+} -containing as well as Ca^{2+}-free media (1–4, 33). Moreover, in these two conditions, a marked depletion of synaptic vesicles (suggesting stimulation of exocytosis possibly coupled with inhibition of vesicle recycling) was revealed by a recently completed morphometric analysis of rat brain synaptosomes exposed to the toxin (unpublished data). Although the influx of Ca^{2+} demonstrated in the present work could explain stimulation of exocytosis in Ca^{2+}-containing media, the data obtained in Ca^{2+}-free conditions appear more problematical. The suggestion (34) that an increased cytosolic Na^+ can induce release into the cytosol of Ca^{2+} from internal stores can be questioned under the present conditions because synaptosomes incubated in the absence of external Ca^{2+} rapidly lose all detectable Ca^{2+}. In addition, it should be mentioned that, in the case of γ-aminobutyrate, release has been reported to occur not by exocytosis but by reverse of the uptake pathway after a decrease in the Na^+-electrochemical potential (35).

CONCLUSIONS

The present study emphasizes the value of considering the synaptosomes as a multicompartment organelle and, in particular, of monitoring the plasma and mitochondrial membrane potentials continuously and independently.

The results obtained by this approach may be explained most simply in terms of an α-latrotoxin-induced increase in the Na^+ and Ca^{2+} conductance of the presynaptic plasma membrane by a mechanism independent from the classical voltage-sensitive Na^+ and Ca^{2+} channels. The resulting collapse of the ion electrochemical potential would lead to transmitter release, although the precise mechanism(s) involved is still undefined. The Ca^{2+} entering the nerve ending as a result of the toxin action is accumulated by the intraterminal mitochondria. Whether the conductance channel activated by the toxin preexists in the membrane or whether it is established by the insertion of the toxin molecule itself into the lipid bilayer (14) is still unclear. In the latter case, however, in order to account for the specific action of α-latrotoxin in nerve terminals, it would be necessary to postulate the membrane insertion to be preceded (and greatly favored) by the toxin binding to high-affinity receptors located in presynaptic membranes (6, 10).

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