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Biochemistry

Use of a lipophilic cation for determination of membrane potential in neuroblastoma–glioma hybrid cell suspensions

([^{3}H]tetraphenylphosphonium$^+$/veratridine/protonophore/monensin/ouabain)

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ABSTRACT **Neuroblastoma–glioma hybrid cells (NG108-15) in suspension accumulate the permeant lipophilic cation [^{3}H]tetraphenylphosphonium (TPP^+) against a concentration gradient. The steady-state level of TPP^+ accumulation is about twice as great in physiological media of low K^+ concentration (i.e., 5 mM K^+/135 mM Na^+) than in a medium of high K^+ concentration (i.e., 121 mM K^+/13.5 mM Na^+). The latter manipulation depolarizes the NG108-15 plasma membrane and indicates that the resting membrane potential ($\Delta\Psi$) is due primarily to a K^+ diffusion gradient ($K^+_{in} \rightarrow K^+_{out}$). TPP^+ accumulation is time and temperature dependent, achieving a steady state in 15–20 min at 37°C, and is a linear function of cell number and TPP^+ concentration (i.e., the concentration gradient is constant). The difference in TPP^+ accumulation in low and high K^+ media under various conditions has been used to calculate mean (±SD) $\Delta\Psi$ values of -56 ± 3, -63 ± 4, and -66 ± 5 mV at 26, 33, and 37°C, respectively. Importantly, these values are virtually identical to those obtained by direct electrophysiological measurements made under the same conditions. TPP^+ accumulation is abolished by the protonophore carbonylcyanide-*m*-chlorophenylhydrazone, whereas the neurotoxic alkaloid veratridine diminishes uptake to the same level as that observed in high K^+ media. In addition, the effect of veratridine is dependent upon the presence of external Na^+ and is blocked by tetrodotoxin. The steady-state level of TPP^+ accumulation is enhanced by monensin, indicating that this ionophore induces hyperpolarization under appropriate conditions. Finally, ouabain has essentially no effect on the steady-state level of TPP^+ accumulation in short-term experiments, suggesting that Na^+,K^+-ATPase activity makes little contribution to the resting potential in these cells. Because many of these observations are corroborated by intracellular recording techniques, it is concluded that TPP^+ distribution measurements can provide a biochemical method for determining membrane potentials in populations of cultured neuronal cells.**

Many of the basic neurophysiological properties that underlie nervous system function have been described and analyzed in neuroblastoma–glioma hybrid cell line NG108-15, thereby establishing this line as an appropriate model system for studies of the nervous system. These properties include: (*i*) generation of action potentials (1); (*ii*) synthesis, storage, and release of the neurotransmitter acetylcholine (2, 3); (*iii*) synapse formation (1, 4); and (*iv*) presence of specific plasma membrane receptor sites for neurotransmitters and neuromodulators, such as acetylcholine, opiates, adenosine, and prostaglandins, which regulate adenylate cyclase (5–9). Because many of these phenomena are clearly related to alterations in the electrical potential ($\Delta\Psi$) across the plasma membrane, correlations between changes in $\Delta\Psi$ and specific biochemical functions take on fundamental importance. Traditionally, measurement of $\Delta\Psi$ in nervous tissue relies on techniques that require impaling selected cells with microelectrodes. However, because this technique is limited by the size of the cells and cannot be applied to populations of cells at large, it is apparent that alternative methods would be of value.

This communication describes the application of a biochemical method for determining $\Delta\Psi$ in cultured neuroblastoma–glioma hybrid cells. The technique involves the use of the permeant lipophilic cation tetraphenylphosphonium (TPP^+) which has been used to measure $\Delta\Psi$ in mitochondria (10), Ehrlich's ascites cells (11), and bacteria and bacterial membrane vesicles.¶ The use of "lipophilic ions" to measure $\Delta\Psi$ across biological membranes was introduced by Skulachev and Liberman (for review, see ref. 12) and their coworkers in the early 1970s, and this class of compounds has been applied to a number of prokaryotic and eukaryotic systems (10–16, ¶). The ions are constructed in such a fashion that they are sufficiently lipophilic to enter the hydrophobic core of the membrane, and they also have the propensity for charge delocalization which allows passive equilibration with $\Delta\Psi$ (17). It should be noted, however, that in no case have $\Delta\Psi$ determinations with lipophilic cations been validated quantitatively by direct electrophysiological measurements. The results presented here provide a strong indication that TPP^+ distribution can be used to determine $\Delta\Psi$ in populations of neuroblastoma–glioma hybrid cells.

METHODS

Cells. The mouse neuroblastoma–rat glioma hybrid clone NG108-15 was used and was grown and maintained as described (18). Harvesting was accomplished by shaking the cells off the culture flask and centrifuging at 250–500 × *g* for 5 min at room temperature. The pellets were diluted at least 1:10 and resuspended in a medium containing 135 mM NaCl or 135 mM choline hydrochloride, 50 mM Hepes adjusted to pH 7.4 with Tris base, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5.5 mM glucose. The suspensions were then centrifuged again, and the cells were resuspended in the same solution to a concentration of 14–30 × 10^6 cells per ml. Suspensions were incubated at 37°C for 10 min prior to use.

Uptake of [^{3}H]TPP^+ and [^{35}S]SCN^-. Reactions were initiated by adding 50 μl of washed cells previously equilibrated at 37°C to 450 μl of a solution containing 2–26 μM [^{3}H]TPP^+ [7.2–210 mCi/mmol (1 Ci = 3.7 × 10^{10} becquerels)] (as bromide salt) or 5 mM [^{35}S]SCN^- (0.75 mCi/mmol) (as sodium salt) in one of the following mixtures: (*i*) "low K^+ medium," 135 mM NaCl/5 mM KCl/50 mM Tris·Hepes, pH 7.4/1.8 mM $CaCl_2$/0.8 mM $MgSO_4$/5.5 mM glucose; (*ii*) "high K^+ medium," same

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Abbreviations: TPP^+, tetraphenylphosphonium$^+$; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

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¶ S. Ramos, L. Patel, and H. R. Kaback, unpublished data.

as low K^+ medium except that 130 mM KCl was used in place of NaCl; and (*iii*) "choline$^+$ medium," same as low K^+ medium except that 135 mM choline·HCl was used in place of NaCl and no KCl was present. Reaction vessels containing these solutions were equilibrated at 37°C prior to addition of cells. After initiation of the reactions, incubations were continued at 37°C for given times, and the reaction vessels were centrifuged in a Brinkman–Eppendorf Microcentrifuge (model 3200) for 1 min. The supernatants were immediately aspirated, and the pellets were resuspended in 1.0 ml of 1% Triton X-100 and transferred to counting vials containing 12 ml of Aquasol for measurement of radioactivity by liquid scintillation spectrometry. All assays were performed in duplicate or quadruplicate, and the values did not vary by more than 10%. Corrections for radioactivity trapped in the extracellular space of the pellets were determined by assaying an aliquot of the supernatant and calculating the total amount trapped from measurements of the intracellular space (see below).

Determination of Intracellular and Extracellular Spaces. Cells washed in choline$^+$ medium (0.5–2 × 10^6 cells in 50 μl) were suspended in 450 μl of an appropriate medium (4°C) containing [^{3}H]H_2O and [^{14}C]sorbitol and immediately centrifuged for 1 min. After the supernatant was aspirated, the total volume of the resulting pellets was determined from the [^{3}H]H_2O content, and the extracellular space was determined from the [^{14}C]sorbitol content. The intracellular space of the pellet was taken as the difference between total and extracellular spaces. The values were within 5% of the intracellular space calculated from microscopic measurement of the average diameter of cells suspended in low K^+ and high K^+ media.

Electrophysiological Measurements. Electrophysiologic experiments were performed by Kathleen Dunlap in the laboratory of G. D. Fischbach (Department of Pharmacology, Harvard University Medical School). NG108-15 cells were washed in choline$^+$ medium, placed in low K^+ medium containing 6.8 mM $CaCl_2$ in plastic bacterial petri dishes, and maintained at 26 or 33°C on the stage of an inverted microscope.‖ Individual cells were penetrated with a microelectrode (R = 50–100 MΩ) connected to a resistance bridge circuit so that it could be used simultaneously for intracellular recording and stimulation (19, 20). Concentrations of perturbants in the vicinity of the impaled cell were increased by pressure ejection of appropriate media containing 6.8 mM $CaCl_2$ and a given perturbant from blunt-tip pipettes (21).

Materials. [^{3}H]TPP^+ (0.4 Ci/mmol) was synthesized by the Isotope Synthesis Group of Hoffmann–La Roche, Inc., under the direction of Arnold Liebman according to methods that will be described elsewhere. ¶ TPP bromide was obtained from K & K. [^{35}S]SCN^- (20.4 mCi/mmol; sodium salt), [^{14}C]sorbitol (213 mCi/mmol), and [^{3}H]H_2O (0.1 Ci/g) were purchased from New England Nuclear. Monensin was generously supplied by J. Berger (Hoffman–La Roche, Inc.). All other materials were of reagent grade and obtained from commercial sources.

RESULTS

TPP^+ Accumulation as a Probe of ΔΨ. NG108-15 cells suspended in a medium containing physiological concentrations of Na^+ and K^+ (i.e., 135 mM Na^+/5 mM K^+) took up TPP^+ rapidly for 5–10 min and achieved a steady-state level of accumulation at 15–20 min (Fig. 1) that was maintained for at least 50 min (not shown). On the other hand, when the cells were suspended in a medium containing a high K^+ concentration (i.e., 13.5 mM Na^+/122 mM K^+), the steady-state level of TPP^+ accumulation was depressed by 50–60%. It is noteworthy that, under the latter condition as well, the steady-state level was maintained for at least 50 min (not shown). Because TPP^+ is readily lost from the cells under various conditions (see below), it is apparent that the plateau levels observed represent steady states rather than conversion of the lipophilic cation into a stable cellular product.

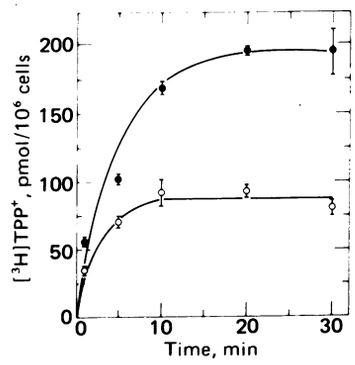

FIG. 1. Time course of [^{3}H]TPP^+ accumulation. NG108-15 cells, washed in low K^+ medium, were suspended in low K^+ (●) or high K^+ (○) medium with 2 μM [^{3}H]TPP^+ (0.21 Ci/mmol) and incubated at 37°C for the times indicated. Each point represents the mean ± SD.

As shown in Fig. 2 *left*, the steady-state level of TPP^+ accumulation by cells suspended in either low or high external K^+ concentrations was linearly related to cell number from 0.7 to 2.4 × 10^6 cells per reaction mixture. Moreover, the difference in TPP^+ accumulation between cells suspended in low and high K^+ exhibited a constant value of 0.7 nmol/10^6 cells at a fixed concentration of TPP^+. In addition, the steady-state level of accumulation was a linear function of TPP^+ concentration from 2 to 26 μM whether the cells were suspended in medium containing low or high K^+ concentration (Fig. 2 *right*). This behavior is consistent with the argument that accumulation of the cation is mediated by a nonsaturable process (i.e., passive equilibration with the ΔΨ across the membrane).

In order to calculate TPP^+ concentration gradients (i.e.,

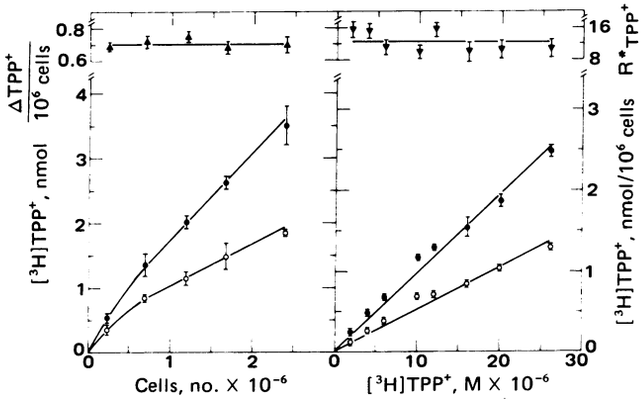

FIG. 2. Effect of cell number (*Left*) and TPP^+ concentration (*Right*) on [^{3}H]TPP^+ accumulation. Cells washed in low K^+ medium were suspended in low K^+ (●) or high K^+ (○) medium for 20 min (37°C). Where cell number was varied, the medium contained 20 μM [^{3}H]TPP^+ (7.2 mCi/mmol); where TPP^+ concentration was varied, assays contained 1.2 × 10^6 cells and [^{3}H]TPP^+ specific activity varied from 15.5–201 mCi/mmol. Each point represents the mean ± SD. $\Delta TPP^+/10^6$ cells = (nmol of TPP^+ at low K^+ − nmol of TPP^+ at high K^+)/10^6 cells. The TPP^+ distribution ratio (R^*_{TPP+}) = ($[TPP^+]_{in}$ at low K^+ − $[TPP^+]_{in}$ at high K^+)/$[TPP]^+_{out}$ (see text for details).

‖ Measurements at temperatures exceeding 33°+C could not be carried out for technical reasons.

Table 1. Membrane potential ($\Delta\Psi$) of NG108-15 cells in suspension

Method	$\Delta\Psi$, mV					
	Low K^+ medium			High K^+ medium		
	37°C	33°C	26°C	37°C	33°C	26°C
Intracellular microelectrodes*	ND	−65 ± 3 (5)	−51 ± 5 (5)	ND	ND	0 ± 1 (2)
[^{3}H]TPP^+ distribution ratio†	−66 ± 5 (12)	−63 ± 4 (6)	−56 ± 3 (6)	0 (12)	0 (6)	0 (6)

Results are expressed as mean ± SD (number of experiments in parentheses). ND, not determined.

* From stable intracellular impalements of medium and large cells in low K^+ and high K^+ media with additional $CaCl_2$ (final concentration, 6.8 mM $CaCl_2$).

† Based on the Nernst equation $\Delta\Psi = -RT/ZF \ln [TPP^+]_{in}^{corrected}/[TPP^+]_{out}$ in which $[TPP^+]_{in}^{corrected} = [TPP^+]_{in}^{low\ K^+} - [TPP^+]_{in}^{high\ K^+}$ (see text for details). The $\Delta\Psi$ based on these measurements is independent of $[Ca^{2+}]$ from 1.8 to 7.5 mM (unpublished results).

$[TPP^+]_{in}/[TPP^+]_{out}$), the intracellular volume under the experimental conditions utilized was determined, (mean values were 2.76 and 2.16 $\mu l/10^6$ cells for cells in low and high K^+ media, respectively). Because intracellular microelectrode recordings from these cells in suspension demonstrated that $\Delta\Psi$ across the plasma membrane is completely abolished in high K^+ medium (Table 1), TPP^+ uptake under these conditions is obviously unrelated to the $\Delta\Psi$ across the plasma membrane. Thus, by subtracting the values obtained for TPP^+ accumulation at high external K^+ concentrations, that component of the total accumulation due to the $\Delta\Psi$ across the plasma membrane can be approximated (i.e., $[TPP^+]_{in}^{low\ K^+} - [TPP^+]_{in}^{high\ K^+} = [TPP^+]_{in}^{corrected}$);†† and dividing by the external TPP^+ concentration yields the concentration ratio (R^*_{TPP+}). The concentration ratio calculated in this manner is constant over the range of TPP^+ concentrations tested (i.e., $R^*_{TPP+} = 12.5 \pm 2.6$). Moreover, when these values are inserted into the Nernst equation ($\Delta\Psi = -61 \log [TPP^+]_{in}^{corrected}/[TPP^+]_{out}$), a $\Delta\Psi$ of −66 ± 5 mV is obtained.

Direct measurement of $\Delta\Psi$ in NG108-15 cells in suspension was performed by using intracellular microelectrodes. The experiments were carried out at 26 and 33°C with cells suspended in low and high K^+ media (Table 1). In low K^+ medium, mean $\Delta\Psi$ values of −51 ± 5 and −65 ± 3 mV were found at 26 and 33°C, respectively. When TPP^+ distribution was measured under identical conditions and $\Delta\Psi$ was calculated as described above, values of −56 ± 3 mV and −63 ± 4 mV were obtained at 26 and 33°C, respectively. In addition, a value of −66 ± 5 mV was obtained at 37°C. Furthermore, $\Delta\Psi$ was abolished in high K^+ medium, as measured by either technique.

Because $\Delta\Psi$ in isolated neurons as well as in NG108-15 cells in suspension is largely the result of a K^+ diffusion potential ($K^+_{in} \rightarrow K^+_{out}$), the effect of external K^+ on the accumulation of TPP^+ and SCN^-, a lipophilic anion, was investigated in detail. As the external K^+ concentration was increased from 5 mM to 121 mM, the steady-state level of TPP^+ accumulation decreased by about 50% (Fig. 3). In contrast, over the same range of K^+ concentrations, the uptake of SCN^- increased almost 2-fold in a reciprocal manner. Because these two permeant lipophilic ions are oppositely charged, the results are consistent with theoretical considerations and provide additional support for the argument that the $\Delta\Psi$ in these cells results from the diffusion of K^+ out of the cell. It is also apparent, however, that approximately 50% of the TPP^+ accumulated by the cells at the steady state is insensitive to external K^+ concentrations that abolish the $\Delta\Psi$ across the plasma membrane. Presumably, part of this pool of TPP^+ represents accumulation of the cation within other internally negative intracellular compartments such as mitochondria that are not sensitive to the extracellular K^+ concentration.

Effect of Ionophores, Veratridine, and Ouabain on TPP^+ Accumulation. NG108-15 cells were allowed to take up the cation to a steady state (i.e., 20 min), at which point a given test compound was added to the reaction mixtures (Fig. 4). When the protonophore carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) was added under these conditions, most of the TPP^+ taken up by cells suspended in low or high K^+ media was lost within 1–2 min. Consistently, intracellular microelectrode recordings from these cells in low K^+ medium demonstrate that CCCP caused almost complete depolarization within 1 min at 33°C (data not shown). Although the $\Delta\Psi$ across the plasma membrane is due primarily to a K^+ diffusion potential, this observation is not surprising because enhanced membrane permeability to cations other than K^+ (in this case, H^+) to a sufficient extent should cause charge neutralization across the membrane. It is also significant that CCCP-induced loss of TPP^+ was observed with cells suspended in high K^+ medium where there is no $\Delta\Psi$ across the plasma membrane. This observation suggests that most of the TPP^+ taken up under these conditions may be due to accumulation within the mitochondrial compartment. In any event, the results demonstrate that the steady-state level of TPP^+ accumulation represents an equilibrium between influx and efflux of the cation.

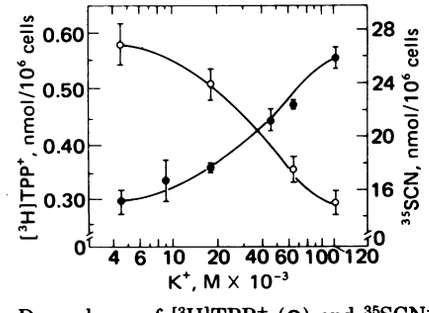

FIG. 3. Dependence of [^{3}H]TPP^+ (○) and $^{35}SCN^-$ (●) accumulation on extracellular K^+. Cells washed with $choline^+$ medium were suspended in medium containing 2 μM [^{3}H]TPP^+ (210 mCi/mmol) or 5 mM $^{35}SCN^-$ (0.75 mCi/mmol) and the indicated concentration choline of K^+ such that $[K^+] + [choline^+] = 121$ mM. Each point represents the mean ± SD.

†† It is important to note that the correction factor $[TPP^+]_{in}^{high\ K^+}$ is an approximation that may underestimate the contribution of intracellular organelles such as mitochondria that have an internally negative $\Delta\Psi$. The concentration gradient of TPP^+ across the membranes of these organelles will remain constant at various cytoplasmic TPP^+ concentrations (i.e., in the presence of high and low extracellular K^+); however, the absolute amount of cation accumulated will depend directly on the cytoplasmic TPP^+ concentration. Thus, the amount of cation accumulated by such organelles will increase proportionately when the $\Delta\Psi$ across the plasma membrane is high (i.e., in the presence of low extracellular K^+) and may not be accounted for quantitatively by correcting for TPP^+ accumulation by cells in high K^+ medium. It is apparent that this qualification is relatively unimportant here because the values obtained correlate extremely well with direct electrophysiological measurements.

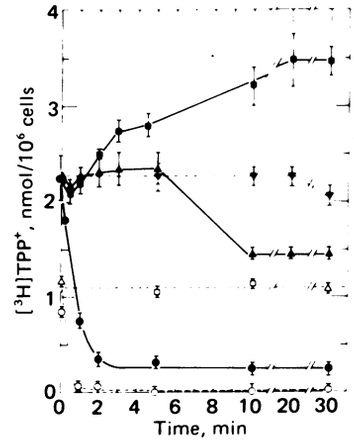

FIG. 4. Reversibility of [^{3}H]TPP$^+$ accumulation. Cells washed in choline$^+$ medium were suspended in low K$^+$ (open symbols) or high K$^+$ (closed symbols) media containing 20 μM [^{3}H]TPP$^+$ (7.2 mCi/mmol). After incubation for 20 min at 37°C (0 time), the following drugs were added: 0.75 μM CCCP (●, ○), 20 μM monensin (■, □), 500 μM veratridine (▲, △), and 10 mM ouabain (▼). In each case, the data obtained were corrected for the value obtained with cells suspended in high K$^+$ medium in the presence of 0.75 μM CCCP (0.68 nmol/10^6 cells).

Monensin is an ionophore that catalyzes electrically neutral exchange of H$^+$ for Na$^+$ primarily across the membrane. Interestingly, when this ionophore was added to NG108-15 cells that had accumulated TPP$^+$ to a steady state in low K$^+$ medium, enhanced accumulation of the cation was observed (Fig. 4). Moreover, intracellular microelectrode recordings from cells under the same conditions demonstrated that addition of monensin produces 20- to 30-mV hyperpolarization (unpublished data), and a similar increase in $\Delta\Psi$ can be calculated from the experiment presented in Fig. 4.

The neurotoxic alkaloid veratridine activates the Na$^+$ ionophore involved in the action potential (22) and depolarizes the plasma membrane of NG108-15 cells because of a marked increase in electrogenic Na$^+$ influx (1). When the alkaloid was added to cells that had accumulated TPP$^+$, there was little or no effect for approximately 5 min, but subsequently TPP$^+$ was lost from the cells and by 10 min the intracellular concentration equaled that observed with cells suspended in high K$^+$ medium (Fig. 4 and Table 2). It is highly significant that the effect of veratridine was dose dependent, that it was not observed when Na$^+$ was omitted from the external medium [i.e., with cells suspended in media containing either high K$^+$ (Fig. 4) or choline$^+$ (Table 2)], and that the loss of TPP$^+$ was mirrored by a reciprocal increase in the intracellular concentration of SCN$^-$. Moreover, when tetrodotoxin was added to the cells prior to veratridine, the effect of the alkaloid was blocked virtually completely. This effect is consistent with the well-known ability of tetrodotoxin to block Na$^+$ influx during the action potential (23, 24).

Because the generation of Na$^+$ and K$^+$ concentration gradients across the plasma membrane of neuronal cells (and thus $\Delta\Psi$) is thought to be due primarily to the action of the Na$^+$,K$^+$-ATPase, the effect of ouabain on TPP$^+$ accumulation was also investigated (Fig. 4). At concentrations of the cardiac glycoside that block Na$^+$,K$^+$-ATPase activity in various rat and mouse cells (1, 25), there was little or no effect on the steady-state level of TPP$^+$ accumulation. Over longer periods of time, however, slow loss of the cation occurred in the presence of ouabain, and by about 1 hr the level of TPP$^+$ in these cells approximated that observed when the cells were suspended in high K$^+$ medium (not shown). Thus, the activity of the pump does not make a significant contribution to $\Delta\Psi$ under these conditions. Furthermore, it appears likely that the $\Delta\Psi$ across the plasma membrane of NG108-15 cells is due almost entirely to a K$^+$ diffusion gradient that is maintained for relatively long periods of time even when the pump is inactivated.

Table 2. Effect of veratridine on [^{3}H]TPP$^+$ and ^{35}SCN$^-$ accumulation in NG108-15 cells

Medium	Additions	% of control* [^{3}H]TPP$^+$	% of control* ^{35}SCN
Low K$^+$	None	100 ± 11 (12)	100 ± 8 (4)
	Verat., 500 μM	50.7 ± 3 (6)	ND
	Verat., 50 μM	76 ± 2 (6)	124 ± 2(2)
	Tetrod., 5 μM	100 ± 5 (4)	ND
	Verat., 50 μM, and tetrod., 5 μM	97 ± 2 (4)	ND
High K$^+$	None	59.9 ± 6 (12)	141 ± 1 (4)
	Verat., 50 μM	59.0 ± 3 (4)	ND
Choline$^+$	None	102 ± 3 (6)	ND
	Verat., 50 μM	111 ± 4 (4)	ND

Fifty microliters of NG108-15 cells (1 × 10^6 cells) washed in choline$^+$ medium was incubated for 20 min (37°C) in 450 μl of the above media containing the indicated components with 20 μM [^{3}H]TPP$^+$ (7.2 Ci/mmol) or 5 mM ^{35}SCN (0.75 mCi/mmol). Verat., veratridine; tetrod., tetrodotoxin.

* TPP$^+$ or SCN$^-$ accumulated by cells in low K$^+$ medium was taken as 100%. Results are presented as mean ± SD (number of experiments in parentheses). ND, not determined.

DISCUSSION

The results presented in this paper provide convincing evidence that the $\Delta\Psi$ present across the plasma membrane of neuroblastoma–glioma hybrid cells in suspension can be monitored by measuring the distribution of the permeant lipophilic cation TPP$^+$ in low and high K$^+$ media. Accumulation of the cation in low K$^+$ medium is presumably the result of accumulation across the plasma membrane as well as accumulation across the membranes of intracellular organelles that manifest an internally negative $\Delta\Psi$. However, because the $\Delta\Psi$ across the plasma membrane of these cells is due almost entirely to a K$^+$ diffusion potential ($K^+_{in} \rightarrow K^+_{out}$), that component of the total accumulation due to the $\Delta\Psi$ across the plasma membrane can be approximated by studying the differential accumulation of TPP$^+$ by cells suspended in low and high K$^+$ media.†† With this approach, $\Delta\Psi$ values obtained from TPP$^+$ distribution studies are remarkably similar quantitatively to those obtained by intracellular microelectrode recording from individual cells under various conditions.

The change in the equilibrium distribution of the permeant anion SCN$^-$ between low and high K$^+$ media has been used (26) to calculate a $\Delta\Psi$ of −48 mV for a population of mouse neuroblastoma N18 cells attached to a surface. This value is consistent with the $\Delta\Psi$, −41 mV, obtained by electrophysiological measurement (26). From the studies reported here, which were carried out with cells in suspension, $\Delta\Psi$ values of −56, −63, and −66 mV were calculated from TPP$^+$ distribution measurements at 26, 33, and 37°C, respectively, and values of −51 and −65 mV were obtained electrophysiologically at 26 and 33°C, respectively. Moreover, NG108-15 cells in suspension also generate evoked as well as spontaneous action potentials (unpublished results). Thus, suspensions of these cells retain many of the electrophysiological properties of intact neurons.

It is important to note that the choice of a lipophilic ion for

measurement of $\Delta\Psi$ may be critical. Although resting potentials can be monitored with SCN^- or TPP^+, distribution measurements with the latter are more sensitive because the cation is concentrated intracellularly. In addition, it is noteworthy that triphenylmethylphosphonium$^+$, another lipophilic cation that has been useful in certain systems (13, 15) is not accumulated by NG108-15 cells, even when used in the presence of tetraphenylboron$^-$ (unpublished results).

The results of experiments with various drugs support the general conclusion that TPP^+ distribution is a valid measure of $\Delta\Psi$ in NG108-15 cells. CCCP, a protonophore that causes a generalized increase in membrane permeability to H^+ in many systems, depolarizes these cells electrophysiologically and induces virtually complete loss of TPP^+ from cells suspended in low or high K^+ medium. In addition, the effect of CCCP on cells in high K^+ medium suggests that most of the TPP^+ taken up under these conditions may be due to accumulation by mitochondria. Veratridine, a neurotoxic alkaloid that depolarizes nerve cells secondarily to a specific increase in electrogenic Na^+ influx (22), causes efflux of TPP^+ to a level observed when the cells are suspended in high K^+ medium. Furthermore, in agreement with its known mode of action, the effect of the alkaloid on TPP^+ accumulation is dependent upon the presence of extracellular Na^+ and is blocked by tetrodotoxin. Ouabain has little effect on the steady-state level of TPP^+ accumulation in short-term experiments, indicating that $\Delta\Psi$ in these cells results almost entirely from a K^+ diffusion gradient that can be maintained for relatively long periods of time in the absence of pump activity.

It is of considerable interest that monensin causes hyperpolarization of the plasma membrane in NG108-15 cells as shown by TPP^+ distribution studies and confirmed electrophysiologically. Because monensin catalyzes the transmembrane exchange of internal H^+ for external Na^+, hyperpolarization could result from either of two effects or a combination thereof, none of which can be resolved from the present data. First, an increase in intracellular Na^+ may stimulate Na^+,K^+-ATPase activity and thus an increase in electrogenic pump activity. Such an effect has been reported as the result of microinjections of Na^+ into neurons in snail ganglia (27, 28). Second, in analogy to bacterial membrane vesicles (29, 30), monensin may cause an increase in $\Delta\Psi$ at the expense of the Na^+ gradient without an effect on the pump in a manner that follows directly from considerations of the dynamics of the electrochemical ion gradients across the cell membrane. Under steady-state conditions, the interior of the cell is negative with respect to the outside and, in addition, there is a Na^+ gradient across the membrane ($[Na^+]_{out} > [Na^+]_{in}$). Given this set of conditions, it is reasonable to suggest that the magnitude of $\Delta\Psi$ is limited, to some degree at least, by the Na^+ gradient (i.e., Na^+ will tend to diffuse into the cell down its concentration gradient and with the electrical gradient, providing a force that will limit $\Delta\Psi$). Therefore, by dissipating the Na^+ concentration gradient in an electrically neutral fashion (monensin catalyzed 1:1 exchange of Na^+ for H^+ under most conditions), a force that is limiting for $\Delta\Psi$ is removed and $\Delta\Psi$ may increase without a corresponding increase in pump activity. By testing the effect of ouabain on the increase in TPP^+ accumulation induced by monensin, it should be possible to differentiate between these alternatives. Finally, correlating possible changes in $\Delta\Psi$, based on TPP^+ distribution, with ligand receptor occupancy, changes in cyclic nucleotide metabolism, and other properties should be of considerable interest.

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