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The K^+ Liquid Ion Exchange Electrode System: Responses to Drugs and Neurotransmitters

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K^+-sensitive liquid ion exchange electrode systems respond with a slow potential change to acetylcholine, choline, anticholinergic drugs, biogenic amines, and glutamic acid. The response threshold has been defined, and in most cases it is at extremely low concentrations (10^{-7}–10^{-5} M). The direction of the potential change varies, but in most instances it is additive to that produced by external K^+. The K^+ electrode system is further sensitive to decreasing pH, within a narrow and possibly physiological range of pH. These findings suggest that small measured changes in extracellular K^+ are biased by the chemosensitivity of the liquid ion exchange electrode system to some compounds of physiological importance.

Key words: liquid ion exchange electrode systems, response threshold, chemosensitivity, K^+ ions

INTRODUCTION

Neuronal activity is associated with transient potassium fluxes, and accumulation of that ion in the extracellular space appears possible [Baylor and Nichols, 1969]. The changes in extracellular K^+ may in turn modulate the behavior of both neurons and glia [Lothman and Somjen, 1975]. The development of liquid cation exchange resins with a large selectivity for K^+ over Na^+, the major extracellular cation, has made it possible to use such resins for K^+-specific microelectrodes to measure both intracellular and extracellular K^+ changes in a variety of preparations [Hnik et al, 1972; Kriz et al, 1974; Kunze and Brown, 1971; Neher and Lux, 1973; Vyskocil and Kriz, 1972; Walker, 1971]. As a result of neuronal activity, extracellular changes of many organic molecules as well as ions may occur. Therefore, neurotransmitter or their precursors may well contribute to the potential change recorded by the K^+ ion-specific electrode system, which are inter-

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preted as specific changes in extracellular K^+ concentration. In fact, such electrodes have been shown to respond to acetylcholine (Ach) at 10^{-3} M [Neher and Lux, 1973], suggesting that responses might be obtained at lower and more physiological concentrations of Ach. Tetraethylammonium (TEA) at 10^{-3}M drastically impairs K^+ electrode function; in fact, a ten-fold increase in the concentration of TEA results in potential changes of 150–200 mV [Neher and Lux, 1973]. The paucity of data on the chemosensitivity of the K^+ ion exchange electrode to organic compounds of physiological significance prompted the present study.

We have looked at the responses of the K^+ ion exchange electrode system to putative amino acid neurotransmitters, such as γ-aminobutyric acid (GABA) and glutamic acid (GA). As representative of the biogenic amines, we have tested the effects of norpinephrine (NE) and serotonin (5HT) on the K^+ liquid ion exchange electrode system. The concentration threshold responses of the K^+ electrode system to various neurotransmitters and drugs have been determined to more carefully evaluate the utility of the K^+ liquid ion exchange electrode system for the measurement of changes in extracellular K^+.

MATERIALS AND METHODS

Single micropipettes were prepared from Simax glass tubing (No. 34502) with an internal diameter of 0.9–1.1 mm, using a horizontal pipette puller. The tip diameter was approximately 1 μm with a large taper angle [Vyskocil and Kriz, 1972]. In the experiments, such fine tips are not necessary because the volume of the chamber used was large enough to accept larger tip electrodes. The tips were then carefully broken against a porcelain plate under microscopic control to produce a final tip diameter in the range of 5–10 μm. The K^+ electrode system consisted of a pair of micropipettes similar in shape and tip diameter; one for the K^+ ion-specific electrode and the other for the indifferent electrode. The procedure for filling of the K^+ liquid ion exchange electrodes has been described in detail by others [Vyskocil and Kriz, 1972; Walker, 1971]. The micropipette tips were siliconized by immersion in a 3% solution of tri-n-butyl-chlorsilane in 1-chlornaphthalene for a short time, allowing the tips to fill by capillary forces to the desired height. The pipettes were baked in an oven at 250°C for 1 hour, and then filled with the liquid K^+ ion-exchanger (Corning 477317). The space above the ion-exchanger was filled with a solution of 0.5 M KCl. The exchanger column height was adjusted (200–1,000 μm) by pressure injection of the 0.5 M KCl solution under microscopic inspection. The indifferent electrode was filled with 150 mM NaCl solution that was the same sodium concentration as the external solution, to minimize their diffusion potentials. Only newly prepared electrodes were used in these studies. The resistance of the K^+ electrodes was approximately 2×10^8 ohm. Using such a relatively large tip diameter, the contribution of tip potentials to the K^+ electrode responses appears to be negligible [Lavalle and Szabo, 1969].

In these experiments, the control medium was Hanks' basic salt solution (pH 7.4) containing 5.5 mM K^+ and 150 mM Na^+, as well as physiological concentrations of Ca^{2+}, Mg^{2+}, Cl^- and PO_4^{2-}. The rationale for choosing this particular medium was that this is the standard tissue culture medium used in our physiological experiments of cultured neurons in vitro [Kuramoto and Haber, 1977]. Calibration solution for the K^+ electrode system contained a constant 150 mM NaCl and a varying concentration of KCl (0–20 mM and 0.5 M). The pH of the Hanks' solution was adjusted with either hydrochloric acid or sodium hydroxide; the amounts of either acid or base needed to adjust the pH did not significantly increase the overall external Na^+. All the various organic substances tested were made up freshly in

Hanks' solution (pH 7.4) and used immediately. The norepinephrine solutions contained a trace of ascorbic acid to minimize heavy metal oxidation; ascorbic acid, per se, had no effect on the responses of the K^+ electrodes. Acetylcholine, choline, atropine, and d-tubocurarine were from Sigma. Norepinephrine, L-glutamic acid, γ-aminobutyric acid, and serotonin were from Schwartz-Mann Biochemicals. L-propranolol was generously donated by Ayerst Laboratories. 1-Chlornaphthaline and tri-n-butyl-chlorsilane were from Aldrich Chemicals and Columbia Organic Chemicals, respectively.

The recording chamber was a glass U-tube (1 mm in inner diameter and 20 cm in length), allowing continuous perfusion of the Hanks' solution at a rate of 1 ml/min with a peristaltic pump. The whole U-tube was surrounded by heated water and the temperature was maintained at 37°C. Test solutions were introduced into the U-tube from one side via the pump and stopcocks. Both the K^+ electrode and the indifferent electrode were put at the other side. A constant fluid level in the recording chamber was maintained by a suction pipette to prevent the potential changes produced by the fluid level changes.

The K^+ electrode and the indifferent electrode were connected via Ag-AgCl wires to a DC unity gain differential amplifier (Analog Devices 42K, input impedance 10^{13} ohm). In order to exclude noise and other electrical artifacts, the recording chamber was grounded with an Ag-AgCl wire. The K^+ electrode responses were amplified and recorded with a pen recorder (Grass 7B) with a low noise level DC amplifier.

RESULTS

The potential measured with the K^+ electrode system in the Hanks' solution was approximately −75 mV. When the Hanks' solution was replaced with a test solution consisting of only 5.5 mM KCl and 150 mM NaCl, no detectable potential changes could be found; this demonstrated that other ions, such as Ca^{2+}, Mg^{2+}, Cl^- and PO_4^{2-}, at physiological concentrations did not affect the K^+ electrode function significantly. This potential measured in the Hanks' solution is referred to in the experiments as the zero potential. All further data are presented as positive or negative changes relative to the potential obtained in the control solution.

When the control solution was changed to solutions containing more than 5.5 mM K^+ and a constant 150 mM $[Na^+]$, positive potential changes took place with a delay of 1 min (Fig. 1). The time course of the positive potential changes was uniformly slow. It took 2–3 min to rise to the steady state maximal potential. When the test solution was changed to the control solution, the positive potential returned to the original zero potential with a similar slow time course observed during the rate of rise. In test solution containing less than 5.5 mM K^+, negative potential changes were observed with a slow time course similar to that of the positive potential changes and these returned slowly to the original zero potential as the external $[K^+]$ concentration returned to 5.5 mM. The shape of the potential change produced by 8 mM $[K^+]$ was approximately the mirror image of that produced by 3 mM $[K^+]$ against the reference zero potential. The responses measured with the K^+ electrode system to decreasing pH and to the application of neurotransmitters and drugs also exhibited a slow time course similar to that seen with changes in external $[K^+]$. The amplitude of the responses recorded with the K^+ electrode system have been measured at 3–5 min after onset of the responses. Both amplitude and time course of each response are approximately equivalent. The same responses could be recorded repetitively with the same K^+ electrode system, through some variation appeared from electrode to electrode. All the responses of the electrodes to $[K^+]$ and drugs tested were highly reproducible.

Figure 1 shows a calibration curve for the K^+ electrode system, with the vertical bars showing the range and reproducibility for 20 individual electrodes. The response of the various electrodes was approximately linear in the range of 3–10 mM $[K^+]$ and most electrodes had a slope of 3 mV/mM $[K^+]$. The electrode variations in the observed responses are very small (less than 2 mV) between 3–10 mM $[K^+]$. However, below 4 mM $[K^+]$ the variability is greatly increased. The potential measured in K^+-free solution (150 mM NaCl) was approximately 30 mV more negative than in the control solution (5.5 mM $[K^+]$).

In the control solution, the response of the K^+ electrode system was altered by decreasing pH from 7 to 5, but not by increasing pH (Fig. 2). No measurements were made at pH values greater than 9 or lower than 5. The polarity of the potential change at pH values below 7 was negative with respect to the potential referred to as zero (5.5 mM $[K^+]$). In experiments not shown in Figure 2, concurrent changes in pH and $[K^+]$ result in additive potential changes measured with the electrodes.

Figure 3 shows the responses of the K^+ electrode system to acetylcholine (Ach). Potential changes of a positive polarity were seen at 10^{-7} and 10^{-8} M, with both the iodide and chloride salts. Increasing concentrations of external Ach result in progressively larger potential changes at a constant 5.5 mM $[K^+]$. For each Ach concentration, a minimum of five individual electrodes was used; it is evident that at higher Ach concentrations (greater than 10^{-5} M) the variability of the electrode response is very large. However, at threshold and possibly more physiological concentrations, the variability is small and reproducible. Acetylcholine (10^{-7} M) results in a potential change equivalent to an increase of 0.5–1.5 mM in external $[K^+]$, whereas at 10^{-5} M Ach the electrode response results in the same potential change as an increase in external $[K^+]$ from 10 to 40 mM.

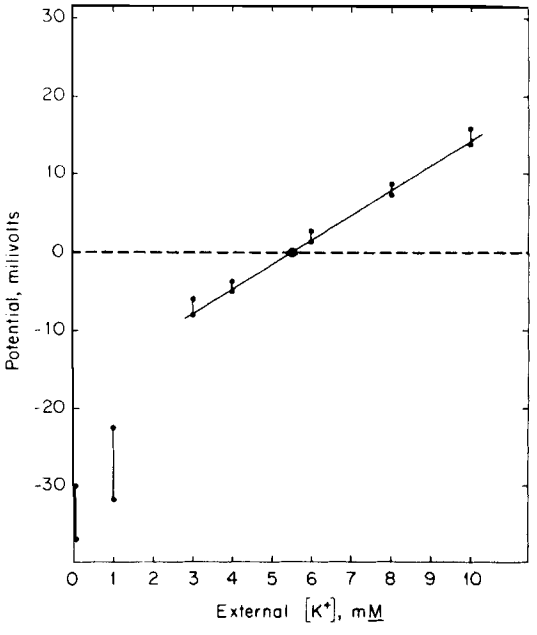

Fig. 1. Calibration curve of the K^+ electrode. Electrode potentials are plotted as a function of increasing external $[K^+]$ in a test solution containing 150 mM Na^+. The vertical bars represent the upper and lower limits of potentials recorded with 20 electrodes.

The Ach response of the K^+ electrode system is further characterized in Figure 4 as a function of $[K^+]$. Curve B in Figure 4 shows the responses at a constant Ach concentration (10^{-5} M). The calibration curve A and the response curve B are approximately parallel, with a constant difference of 30 mV. Though not shown, similar additive effects of Ach and $[K^+]$ were obtained at lower concentrations of Ach. In some electrodes, however, extreme potential changes by 10^{-5} M Ach were seen at 10 mM $[K^+]$. Thus if the electrodes were carefully selected, the responses to $[K^+]$ and Ach behave independently within the narrow range of K^+ concentrations (3–10 mM). The electrode responses produced by concurrent application of $[K^+]$ and Ach at low concentration result in roughly additive responses.

Choline, the precursor of Ach, produces a potential change of the same polarity as Ach, although at a higher threshold (10^{-5} M). In data not shown here, the effects of choline and $[K^+]$ were additive only within a very narrow range of $[K^+]$ and are often minimal at higher $[K^+]$.

The sensitivity of the K^+ electrode system to several neurotransmitters and pharmacological agents is summarized in Table I. The polarity of the potential change, positive or negative, refers to the standard potential measured in Hanks' solution (5.5 mM $[K^+]$, 150 mM $[Na^+]$ and pH 7.4). The threshold is defined as the molar concentration resulting in a potential change greater than 1 mV.

The nicotinic and muscarinic cholinergic receptor blockers, D-tubocurarine (dTc) and atropine, resulted in potential changes of the electrodes of opposite polarity. Ach and dTc gave positive potential changes, both at a threshold of 10^{-7} M. The threshold for atropine was at 10^{-5} M, in contrast to the lower thresholds seen with Ach and dTc.

The concentration dependent responses of the K^+ electrode system to some primary amines, choline and glutamic acid (GA), are shown in Figure 5, and the threshold for these compounds is listed in Table I. The biogenic amines, norepinephrine (NE) and serotonin

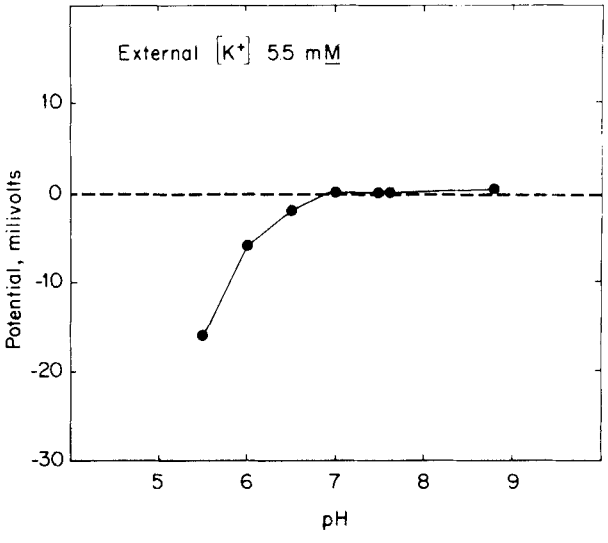

Fig. 2. The response curve of the K^+ electrode to changes in external pH. Responses of the K^+ electrode in mV, plotted as a function of varying pH (5–9) of a Hanks' solution, at a constant 5.5 mM K^+. Zero refers to the potential observed at a constant 5.5 mM K^+.

(5HT), were effective at 10^{-5} M, resulting in opposite changes of polarity in measured potential (Table I). For NE and 5HT the responses could be incorrectly interpreted as a decrease on one hand and an increase on the other in $[K^+]$. The responses to 5HT and NE are shown in Figure 5, curves C and D, respectively. In results not shown here, NE, at threshold concentrations and above, results in potential changes that are completely additive in the range of 1–10 mM $[K^+]$.

L-propranolol, the β-receptor blocker, was more effective than NE, with a threshold of 10^{-6} M, and a polarity change interpretable as an increase in $[K^+]$ (Table I). The concentration response curve to paramethoxyphenylethylamine (PMPEA), which is known to modify monosynaptic spinal reflexes [Walker et al, 1972], results in potential changes dramatically different from those produced by NE (Fig. 5, curve A). The threshold to 5HT is 10^{-5} M (Table I), and the concentration dependence of the electrode response is shown in Figure 5, curve C. The polarity of the 5HT response is opposite to that seen with NE. 5HT and PMPEA effects are additive with those of external $[K^+]$ in the range of 1–10 mM. Glutamic acid (GA) is a possible excitatory transmitter in the mammalian CNS [Roberts and Hammerschlag, 1972]. The decarboxylation product of GA, γ-aminobutyric acid (GABA), is a major inhibitory transmitter in the mammalian [Saito et al, 1976] and crustacean nervous systems [Otsuka et al, 1966]. GA at 10^{-5} M results in a negative potential change, whereas GABA was not effective at concentrations as high as 10^{-4} M (Table I). The effects pf GA (Fig. 5, curve E) are indentical to those of NE with respect to both magni-

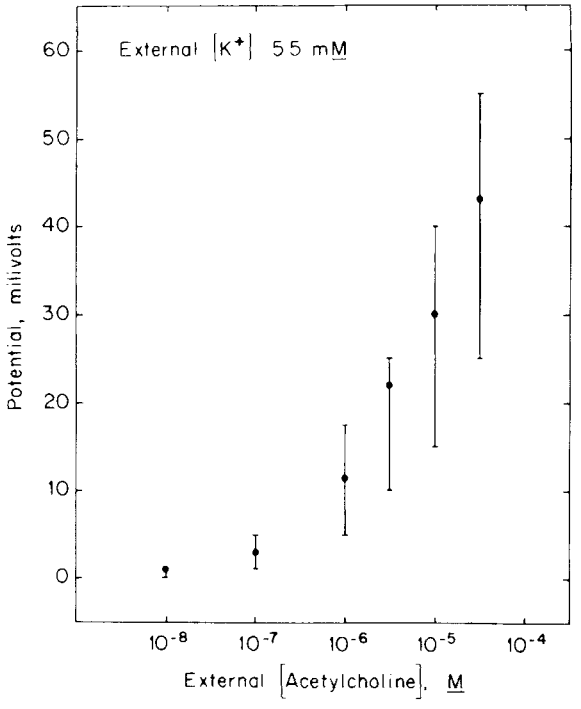

Fig. 3. Response of the K^+ electrode to external acetycholine. The responses of the K^+ electrode are plotted in mV as a function of the external Ach concentration [M]. The vertical bars represent the range of responses obtained with a minimum of five individual electrodes at each Ach concentration.

tude and polarity. The electrode response to GA was additive with those of external [K^+] (1–10 mM).

As a whole, all potential changes produced by the putative neurotransmitters and pharmacological agents tested at concentrations lower than 10^{-5} M resulted in additive potential changes when measured with the K^+ liquid ion exchange electrode system.

DISCUSSION

It has been previously shown by others that the K^+ electrode is sensitive to Ach [Neher and Lux, 1973]. We have extended these observations and characterized more fully the chemosensitivity of the K^+ electrode system for organic molecules of physiological significance. Specifically, neurotransmitters and pharmacological agents may interfere with the K^+ liquid ion exchange resin function. Thus if single specific sites on the resin react to K^+ and other agents, some competitive or cooperative interaction should be observed in the electrode responses to the mixture of K^+ and other ions. Such could be seen in the re-

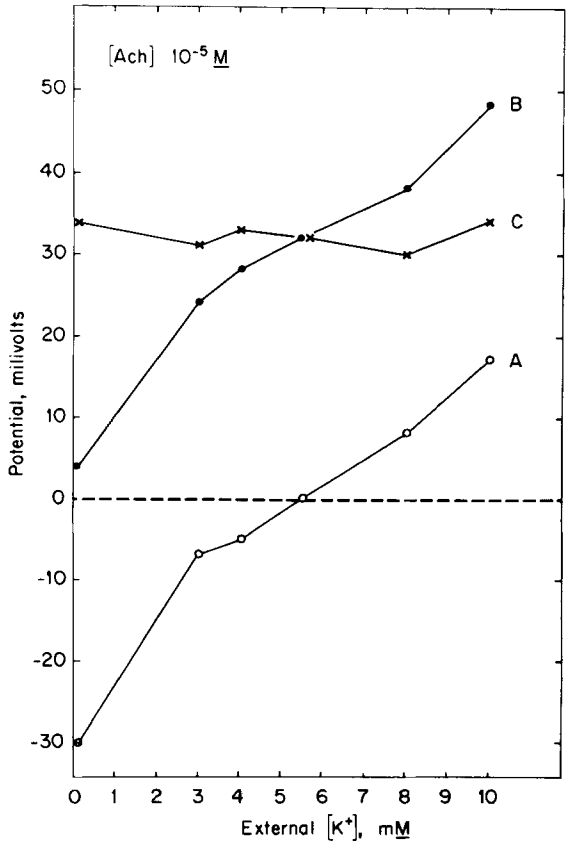

Fig. 4. Additive effects of external acetylcholine and K^+. Curve A is the standard calibration curve for the K^+ electrode (zero refers to potential observed at 5.5 mM K^+). Curve B represents responses of the electrode to alterations in external [K^+] in the presence of a constant concentration of Ach (10^{-5} M). Curve C represents the potentials of B minus A.

TABLE I. Sensitivity of the K^+ Electrode to Neurotransmitters and Pharmacological Agents

Test compound	Responses threshold	Direction of potential change
Acetylcholine (Ach)	10^{-7}–(10^{-9})	+
Choline	10^{-6}	+
D-Tubocurarine (dTc)	10^{-7}	+
Atropine	10^{-5}	–
Norepinephrine (NE)	10^{-5}	–
δ-Propranolol	10^{-6}	+
Paramethoxyphenylethylamine (PMPEA)	10^{-6}	+
5-Hydroxytryptamine (5HT)	10^{-5}	+
Glutamic acid (GA)	10^{-5}	–
γ-Aminobutyric acid (GABA)	10^{-4}	None

Responses of the K^+ electrode were measured at 37°C in Hanks' solution containing 5.5 mM K^+.

sponses to Ach and choline. However, at lower concentrations (less than 10^{-5} M), the effects were reproducible and seem to be additive with those of [K^+] within a narrow range of 3–10 mM. Moreover, responses measured by the electrode system to K^+ and organic molecules appear as independent responses of the electrode.

Tip potentials and diffusion potentials often produce some difficult problems for measurements with glass microelectrode systems [Lavalle and Szabo, 1969]. The K^+ electrode system employed for this experiment was similar to that used commonly by others [Neher and Lux, 1973; Vyskocil and Kriz, 1972; Walker, 1971]. The K^+ electrode and the indifferent electrode used were made of the same type of glass micropietttes with relatively large tip diameters (5–10 μm). The indifferent electrodes were filled with 150 mM NaCl solution, that being the same sodium concentration as in the external solution. Therefore, the contribution of tip potentials and diffusion potentials to the electrode responses can be considered as minimal. Nevertheless, the data do indicate the possibility of some artifactual potential changes contributing to the K^+ electrode responses to solutions containing organic agents. These considerations bias the use of such electrodes to assess extracellular steady-state K^+ ion concentration changes in vivo and in vitro even though the selectivity of the resin for K^+ ion is fairly high.

The selectivity ratio of the resin for K^+ ion over Na^+ was 50 or greater. The calibration curve (Fig. 1) was roughly linear within the narrow range of 3–10 mM [K^+]. Most of the electrodes had a slope of 3 mV/mM [K^+]. The electrode variation in the observed responses was very small (less than 2 mV). Thus the electrodes used were fairly good K^+ electrodes, though not ideal K^+ electrodes for measurement of only inorganic ions in the absence of organic ions. On the other hand, small variation in the reference potential (0 mV in this study) often appeared from electrode to electrode within a range of 3 mV. The slope of the response appears to flatten from 3–5 mM [K^+] and rise more steeply above 8 mM [K^+]. This may be due to variations of the reference potential, in that all potential changes were measured against the reference potential, which per se could vary within a 3 mV range. However, these variations were very small and do not greatly affect the measurements of much larger potential changes.

The present studies were focused at the extracellular steady-state responses of the K^+ electrode system such as might be seen in measurements of K^+ ion concentration changes in tissue culture media. Thus only maximal values of steady-state responses were recorded and any real differences in the speed of the electrode responses were not recorded. The

slow time course of the electrode responses may be caused by the slow concentration changes of solutions in the recording chamber. The steady-state responses of the electrodes used were all reproducible and all returned to zero, the original baseline (zero potential), when test solutions were replaced with the control medium (Hanks').

The electrode responses to a variety of putative neurotransmitters and drugs that are known to affect synaptic transmission are given in Table I. The response threshold for Ach was as low as 10^{-8} M. Whether this concentration of Ach approaches that which occurs extracellularly in the CNS is conjectural; however, such a concentration is within the range of Ach concentrations used in bath applications or those that might be obtained by iontophoretic application of Ach. It is interesting to note that the measured concentrations of Ach in human epicortical, ventricular, and lumbar cerebrospinal fluids (CSF) are 1.2, 0.5, and 0.2×10^{-6} M, respectively [Grossman et al, 1975].

Choline, the precursor of Ach, likewise interferes with K^+ electrode function. The response threshold is 10^{-6} M, a concentration roughly equivalent to that which has been measured by us in human ventricular fluids, CSF, and plasma [Grossman et al, 1975]. This concentration threshold is analogous to the Km reported for the high affinity transport system for choline in brain synaptosomes [Yamamura and Snyder, 1972] and in cultured neurons and glia [Hutchison et al, 1976].

Previously Neher and Lux [1973] reported that the K^+ electrode was chemosensitive to a variety of substituted quarternary amines at 10^{-3} M, in the presence of external 10

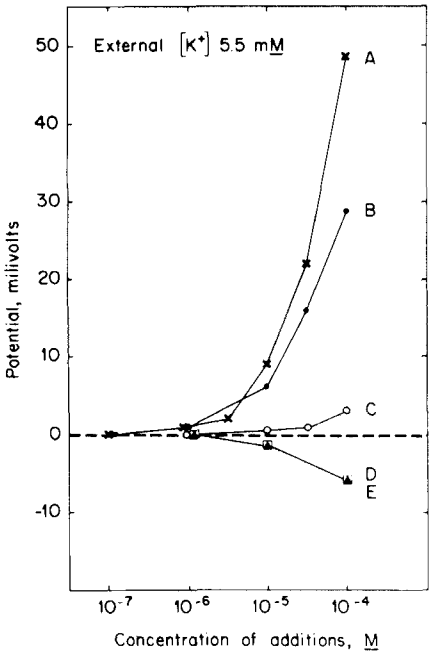

Fig. 5. Responses of the K^+ electrode to primary amines, glutamic acid, and choline. (A) Paramethoxyphenylethylamine (PMPEA), (B) choline, (C) serotonin (5HT), (D) norepinephrine (NE), and (E) glutamic acid (GA). Curves A–E represent responses of the electrode to varying concentrations of test substances measured in a standard Hanks' solution, containing 5.5 mM K^+. Zero refers to the potential observed at 5.5 mM K^+.

mM K^+. However, the combined K^+ and Ach response of the electrode was not shown in detail. We observed that the Ach responses of the K^+ electrode system were additive to those of $[K^+]$ within a narrow range of K^+ concentrations (3–10 mM). The results shown were obtained at an exaggerated concentration of Ach (10^{-5} M); qualitatively similar results are obtained at threshold concentrations of Ach (10^{-7} M).

The response threshold of the K^+ ion exchange electrode to 5HT and NE is 10^{-5} M, although the potential changes observed are of opposite polarity. L-propranolol, a commonly used β-receptor blocker, results in a potential change of the electrode at pharmacologically meaningful concentrations. It is difficult to assess the physiological significance of these extracellular amine concentrations, though identified serotonergic leech neurons contain far higher levels of 5HT [McAdoo and Coggeshall, 1976]. It is likely that intracellular concentrations of NE in adrenergic neurons exceeds by far the response threshold of the K^+ electrode to NE. Furthermore, it is plausible to suggest that the concentrations of both 5HT and NE or other neurotransmitters is higher in nerve terminals than in cell bodies. For example, the calculated concentration of GABA in Purkinje nerve terminals approaches 10^{-1} M [Fonnum and Walberg, 1973], as opposed to the measured levels of 3–6 mM in Purkinje soma [Obata, 1976].

To what extent the possibly higher intracellular concentrations of NE or 5HT translate into significant extracellular concentrations obtained during sustained release at aminergic synapses is conjectural. The intracellular responses of the K^+ electrode are probably not altered by the biogenic amines, as the bulk of the intracellular transmitter store is sequestered in a vesicular storage pool.

Paramethoxyphenylethylamine (PMPEA) is an indirectly acting sympathomimetic amine that modifies monosynaptic spinal reflexes [Walker et al, 1972], produces profound behavioral effects per se, and interacts with both serotonergic and noradrenergic systems [Ashkenazi and Haber, 1975]. PMPEA exerts its threshold effect at a concentration equivalent to that which blocks the uptake of 5HT by a number of neural preparations in vitro [Haber et al, unpublished]. As an o-methylated derivative of phenylethylamine, which is present in mammalian CNS tissue, it may be a model compound for studying the effects of o-methylated metabolites of the catecholamines on responses of the K^+ electrode.

Two putative amino acid neurotransmitters were tested: glutamic acid (GA) and its decarboxylation product, γ-aminobutyric acid. The response threshold to GA is 10^{-5} M, which is significantly lower than the overall brain GA concentrations. In that glutamic acid is also present in significant amounts in the plasma, the additive responses of GA and K^+ may lead to incorrect assessments of K^+ measured in vivo, ie, during spreading depression [Grossman et al, 1975]. It is probable that the K^+ electrode responds in a similar manner to aspartic acid, a second putative dicarboxylic amino acid neurotransmitter. GABA is not effective even at 10^{-4} M and possible effects at much higher concentrations are of doubtful significance.

The K^+ electrode response is altered by an acid pH change (7–5.5). Therefore, physiological changes in pH, such as are likely to occur in acidosis, or lactate accumulation in muscle are likely to lead to incorrect measurement of K^+ measured by the K^+ electrode system.

Taken together, these results suggest that the K^+ liquid ion exchange electrode system has a far lower threshold sensitivity to molecules of physiological significance, other than K^+, than had been suspected to date. This chemosensitivity to neurotransmitters and drugs limits the utility of these electrodes to measurements of extracellular steady-state K^+ concentrations in vivo and in vitro to those situations when adequate recalibration of the elec-

trode is possible. It is suggested that measurement of extracellular K^+ changes by such electrodes would be less biased for in vitro systems, as in tissue culture, where the composition of the media can be adequately defined.

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