# Ammonium and Chloride Extrusion: Hyperpolarizing Synaptic Inhibition in Spinal Motoneurons

Abstract. *The reversal potential of postsynaptic inhibition shifts toward resting membrane potentials in cat spinal motoneurons after intravenous infusion of ammonium salts (1 to 3 millimoles per kilogram of body weight). Simultaneously, the depolarizing action of intracellularly injected chloride ions on the inhibitory membrane is enhanced and recovery therefrom is prolonged. Passive membrane properties remain unaltered. The results indicate a blocking of active extrusion of chloride which normally maintains a high ionic gradient for hyperpolarizing inhibition.*

Systemic application of ammonium salts in dosages in the range of millimoles per kilogram of body weight and below can seriously impair central nervous functions and induce epileptiform seizures (*1*). Accumulation of ammonia in a comparable range of concentrations is reported to be the major change in intracerebral nitrous constituents in many different epileptogenic metabolic disturbances (*2*). A previous study (*3*) was designed to clarify the effect of $NH_4^+$ on neuronal functions. It revealed that $NH_4^+$ acts predominantly on postsynaptic inhibition. Inhibitory transmission is preserved, but inhibitory potentials (IPSP's) are produced which are greatly reduced at normal resting potentials. The effect is highly sensitive to external and systemic applications of $NH_4^+$. It results from a reversible depolarizing shift of

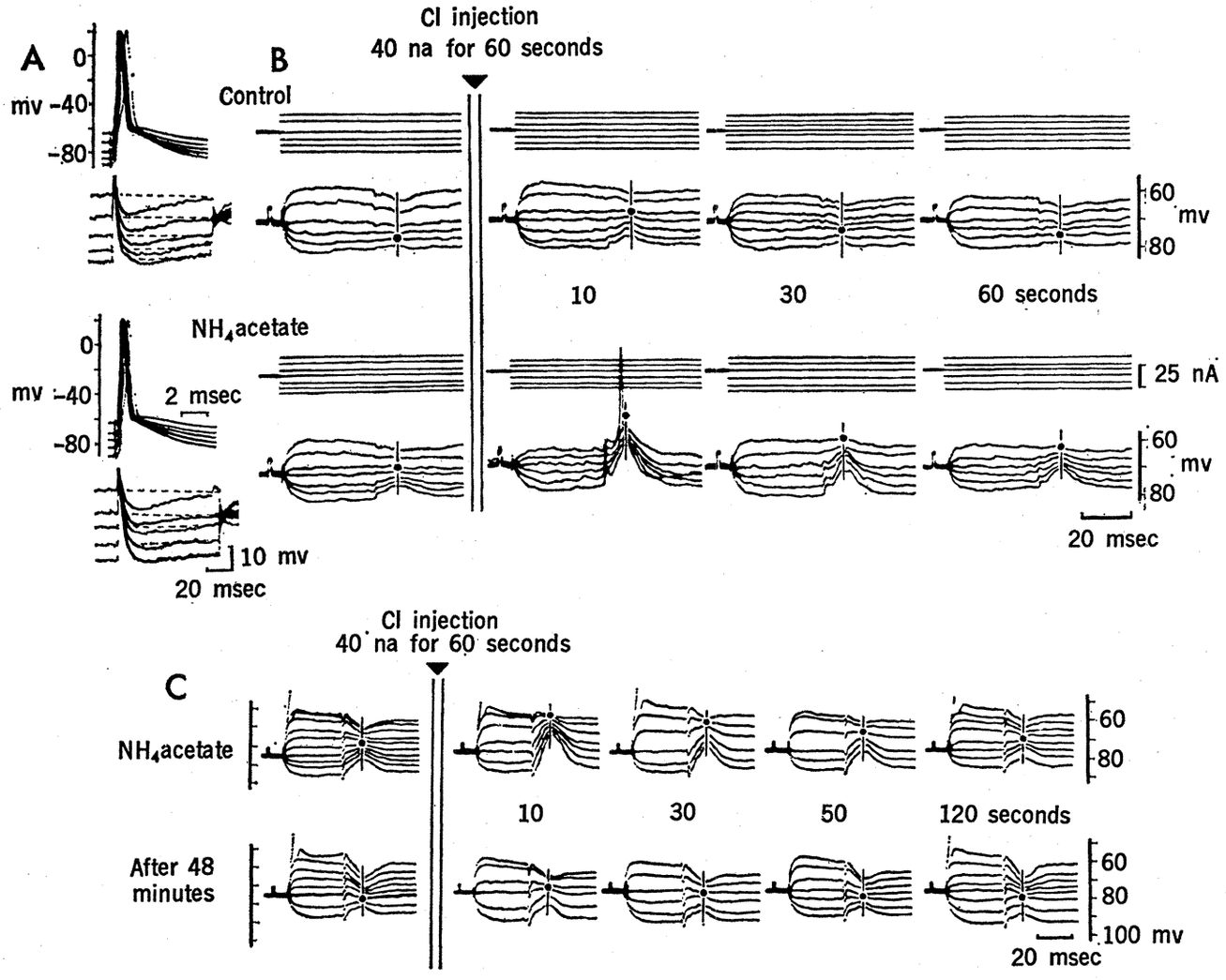


Fig. 1. Effect of intravenous infusion of ammonium acetate (2.6 mmole per kilogram of body weight) on the antidromic action potential (A) and on the IPSP (B) of a lumbar motoneuron. The action potentials in (A) were displayed with different time bases and amplifications. Upper parts of the displays show the superimposed spikes in an expanded section of the lower part (spikes truncated there). Dashed lines indicate membrane potential slopes without action potentials during transmembrane current steps until the end of the step. The series in (B), control, shows (on top) superimposed transmembrane current steps and resulting membrane potentials (below) with elicited IPSP's, shortly before and after $Cl^-$ electrophoresis at indicated times. The compound IPSP in (B) consists of a direct part of short latency followed by a larger polysynaptic part. Vertical lines indicate times at which IPSP's are evaluated, the dots giving the reversal potentials which are similar for the direct IPSP. The series is repeated after infusion of ammonium salt had produced the depolarizing shift of the IPSP reversal point (first part of the series B, $NH_4$ acetate). A spike is elicited by the depolarizing IPSP 10 seconds after injection of $Cl^-$. (C) A similar sequence of antidromic IPSP's at varied membrane potentials in another motoneuon during infusion of ammonium acetate (2.8 mm/kg) and after recovery. The time constants of recovery from injections of $Cl^-$ increased in the presence of $NH_4^+$ from 19 to 36 seconds in (B) and from 21 to 44 seconds in (C).

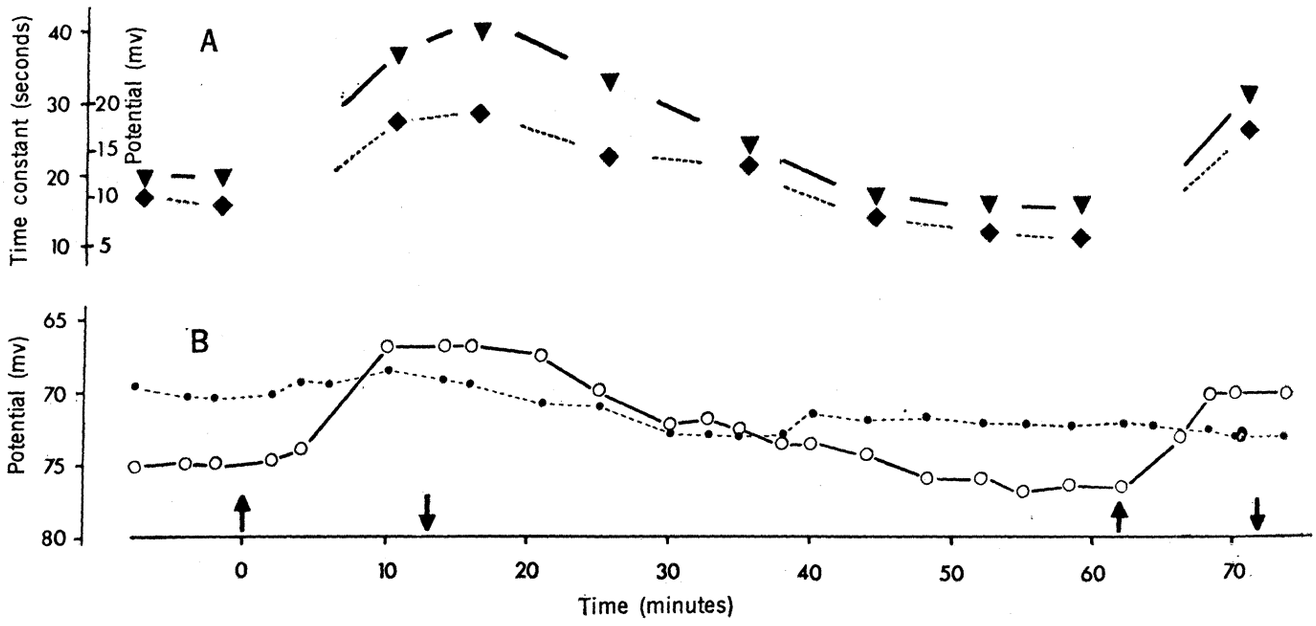


Fig. 2. Survey of time course of the changes produced by infusion of ammonium ion (within arrows) on the IPSP and on the $E_{IPSP}$ recovery process after $Cl^-$ was injected into a motoneuron. In (A) time constants of recovery of $E_{IPSP}$ from injections of chloride are plotted (triangles). Squares indicate $E_{IPSP}$ shifts which are determined within 5 to 10 seconds after termination of $Cl^-$ injections. Values of resting potentials (dots) and steady-state $E_{IPSP}$ (open circles) are given in (B). An initial stabilization period of recording is omitted. The cell was lost shortly after the second infusion of $NH_4^+$ which produced a similar effect on the $E_{IPSP}$ while synaptic effectiveness (slope of IPSP dependence on membrane potential) was then reduced by about one half.

the equilibrium potential of postsynaptic inhibition ($E_{IPSP}$) toward resting membrane potentials. Especially with extracellular electrophoresis, it was apparent that the action is not controlled by diffusion but is indirect because the $E_{IPSP}$ shift developed with a time course of minutes. A change in the ionic conditions of inhibitory electrogenesis appeared likely since $E_{IPSP}$ represents an electrochemical potential resulting from the concentration gradient of those ions which can permeate the activated inhibitory membrane (*4*). The hypothesis was offered that $NH_4^+$ acts in blocking an outwardly directed chloride extrusion mechanism (pump) which normally maintains an electrochemical $Cl^-$ potential higher than resting membrane potential. During this blockade, $Cl^-$ would be redistributed across passive membrane channels according to resting membrane potentials. The hypothesis of a change in chloride extrusion kinetics induced by $NH_4^+$ can be tested by a comparison of the action of injected chloride ions on $E_{IPSP}$ in the normal state with that during application of $NH_4^+$.

The IPSP's of cat spinal motoneurons were elicited by electric stimulation of hindlimb muscle nerves or ventral roots. Double-barreled microelectrodes were used for electrophoresis and simultaneous recording. The recording barrel was filled with 1.5*M* potassium citrate (85 percent) and KCl (15 percent). The other barrel, employed for electrophoresis and other current application, contained 0.5*M* KCl. The animals were decerebrated after an initial dose of pentobarbital (30 mg/kg), immobilized with gallamine triethiodide (Flaxedil), and artificially ventilated (*3*). Ammonium acetate was applied by intravenous infusion. Secondary effects of this convenient kind of application are short-lasting and moderate bradycardia and changes in blood pressure. More conspicuous is a reduced responsiveness to stimulation of afferent nerves, which can last for several minutes. This was observed with inhibitory as well as with excitatory synaptic potentials. A reduced inhibitory action was deduced from the decreased dependence of IPSP sizes upon membrane potentials during application of current steps. A presynaptic alteration has been held responsible for this observation (*3*); such an alteration might be blocking of axonal conduction similar to that caused by potassium. This effect is secondary to the changes in $E_{IPSP}$ and could be greatly reduced or avoided by low infusion rates, between 0.2 and 0.8 ml/min, with a 10 percent ammonium acetate solution of neutral *p*H. At a dosage of 1.5 to 3 mmole per kilogram of body weight the IPSP's are virtually abolished at resting potentials which in general show no significant alterations (Figs. 1 and 2). This is true except during an initial period of several minutes when frequent spontaneous synaptic activity and occasional firing is encountered in many motoneurons. Since the effect of infused $NH_4^+$ is quite long lasting (Fig. 2), it was necessary to assure stable recording situations and, therefore, to prolong the control periods to at least 20 minutes.

A standard transmembrane electrophoretic current of 40 na was applied for 60 seconds to inject chloride ions intracellularly. For controlled application of current we used a floating amplifier configuration which allows current injection with an accuracy of 5 percent for electrode resistances up to 200 megohm (*3*). In each cell (*3*), two to five control injections were made and their effects on the IPSP were compared with the changes evoked by the same standard injections during and after (within 20 minutes) infusions of $NH_4^+$ of 1.6 to 3.2 mmole/kg. In six cells of the sample, further controls could be made when the effect of $NH_4^+$ had completely worn off, 40 to 80 minutes after termination of infusion. These control data were similar

to those obtained before ammonium was infused. In a number of cases, the cell was lost during infusion of $NH_4^+$ but another stable penetration and recording was possible during this period, permitting a complete evaluation of the effects of injecting $Cl^-$ until recovery from $NH_4^+$. The IPSP equilibrium potentials after injection of $Cl^-$ were determined by plotting the size and sign of the IPSP's against the membrane potentials which were varied in quick succession by transmembrane current steps 80 msec long with intervals of 0.7 second. Determinations of $E_{IPSP}$ from sampled data over 5-second periods were repeated every 10 seconds. Examples are given in Fig. 1, B and C.

Both the magnitude of the depolarizing $E_{IPSP}$ shift and the time course of its recovery after injection of $Cl^-$ showed clear differences. The $E_{IPSP}$ shift (after the injection of $Cl^-$ was terminated) increased by a factor of $2.0 \pm 0.45$ (standard deviation), and the recovery time course was prolonged $1.9 \pm 0.2$ times in the individual motoneurons during and shortly after infusion of $NH_4^+$. It is noteworthy that the $E_{IPSP}$ shift was measured from a steady-state level which was already reduced in the presence of $NH_4^+$. In most cells this level was not at resting potential, but slightly positive to it. As judged from previous data (*3*) this appears to be due to $Cl^-$ leakage from the electrophoresis electrodes. Figure 2 gives a typical example of the time course of the parallel changes in the $E_{IPSP}$ and in the effects of $Cl^-$ injection. The mean time constant for the exponential recovery from raised intracellular $Cl^-$ concentration was $22.0 \pm 4.2$ seconds for all controls with 14.5 and 28 seconds the lowest and highest values. During and shortly after infusions of $NH_4^+$ an average of $43.8 \pm 12.5$ seconds for the recovery time constant from $Cl^-$ injections was found. Extreme values were 30 and 71 seconds.

Because the IPSP hyperpolarization in cat spinal motoneurons was assumed to be the result of a synaptically induced simultaneous $K^+$ conductance (*4*), it was of interest to study the changes produced by infusion of $NH_4^+$ on the afterhyperpolarization of the antidromically evoked spike. This was done on motoneurons which showed no significant antidromic IPSP's according to checks with $Cl^-$ injections and, therefore, probably only small if any distortion of this phase of the action potential which is determined by $K^+$ conductance (*4, 5*). The equilibrium (zero) point of the afterhyperpolarization, as determined by plots of its size against varied membrane potentials, showed a small depolarizing shift (of 2 to 4 mv) during infusion of $NH_4^+$ (2 to 3 mmole/kg) (Fig. 1A). This change can be anticipated from the known ability of $NH_4^+$ to partially substitute for external $K^+$ (*6*). However, a hypothesis which attributes the shift of the $E_{IPSP}$ toward resting potential primarily to changes in the $K^+$ potential has no sufficient basis. Obviously, such an explanation would require a change (of 20 to 30 mv) in the equilibrium potential of the spike afterhyperpolarization nearly ten times larger, that is, from its normal value of about 95 mv to resting potentials of around 70 mv.

In this study, the reversal potential of the IPSP was used as an indicator of the electrochemical gradient of the relevant ions which generate the IPSP. A further assumption derived from the independence principle (*5*) was that the possible dependence of IPSP conductance on other ions (for example, $K^+$) is constant when the internal $Cl^-$ concentration is raised by test injections (*4*). It follows then that the determinations of the recovery time after $Cl^-$ injection reflect the kinetics of chloride extrusion across the motoneuronal membrane. The changes in chloride extrusion obviously parallel the changes in $E_{IPSP}$ produced by infusion of $NH_4^+$ (Fig. 2). However, the assumption that both changes occur at passive membrane sites of $Cl^-$ permeability appears unlikely. A block of passive $Cl^-$ channels, if it were the cause of prolonged $Cl^-$ extrusion, cannot explain the simultaneous leveling of $E_{IPSP}$ with resting membrane potentials. On the other hand, this equilibration cannot be ascribed to an increase in passive membrane conductivity for $Cl^-$ which may shunt an electrochemical gradient for hyperpolarizing inhibition. Such an assumption is incompatible with the observed prolonged redistribution of $Cl^-$ during application of ammonium. Moreover, evidence for changes in resting membrane properties, for example in neuronal input resistances and electrotonic time constants, is lacking (*3*). This speaks against an assumption of changes in passive membrane characteristics to which $Cl^-$ conductance should contribute (*4*). The considerable shifts of $E_{IPSP}$ without obvious effects on passive electric membrane characteristics deserve some comment. It is possible that the resting membrane $Cl^-$ conductance is rather small and that the redistribution of internal $Cl^-$ may be governed by subsynaptic compartments as recently proposed for lobster muscle (*7*). The available evidence suggests the blockage of an active chloride extrusion mechanism by ammonium ion as originally proposed (*3*). The significantly prolonged time constants (30 to 70 seconds) may represent the removal of excess $Cl^-$ across remaining passive membrane sites. An active chloride extrusion will speed up the extrusion process if it is dependent on concentration (*8*), and will normally maintain a $Cl^-$ gradient higher than resting potential. Accordingly, the synaptically induced $Cl^-$ conductance should be hyperpolarizing.

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### References and Notes

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8. An additional removable active extrusion process which operates at constant rates independent of concentration is not likely. Such a model fails to account for the observed changes in $E_{IPSP}$ recovery times, although it could explain the changes in $E_{IPSP}$ shifts after $Cl^-$ injections. A suitable model is an active $Cl^-$ extrusion mechanism dependent upon concentration in parallel with passive redistribution of $Cl^-$ across the resting membrane. Analog computer simulation revealed that an additional extrusion process of first-order as well as of second-order kinetics can fit the data within experimental error. For estimating the membrane permeability for $Cl^-$ it is also important to consider the neuronal geometry and, in particular, to account for intradendritic diffusional fluxes which arise from the probably somatic injection site.
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