Postsynaptic Inhibition: Intracellular Effects of Various Ions in Spinal Motoneurons

Abstract. *Long-lasting depolarizing shifts of the electromotive force of postsynaptic inhibition occurred after intracellular injection of ammonium ions, basic amino acids, hydrogen ions, and some bivalent heavy-metal ions. These substances act on specific postsynaptic membrane sites.*

After intracellular iontophoresis of radioactive glycine and cupric ions, useful for intravital labeling (*1*), long-lasting depolarizing shifts of the inhibitory postsynaptic equilibrium potential (E_{IPSP}) occur in cat spinal motoneurons. These findings are unexpected for two reasons. (i) The amino acid seems to be a normal constituent of the intracellular free amino acid pool, and (ii) the effect of glycine is different if applied extracellularly, where it acts like an inhibitory transmitter (*2*). The effects could not be anticipated from present theories of the postsynaptic inhibitory mechanism.

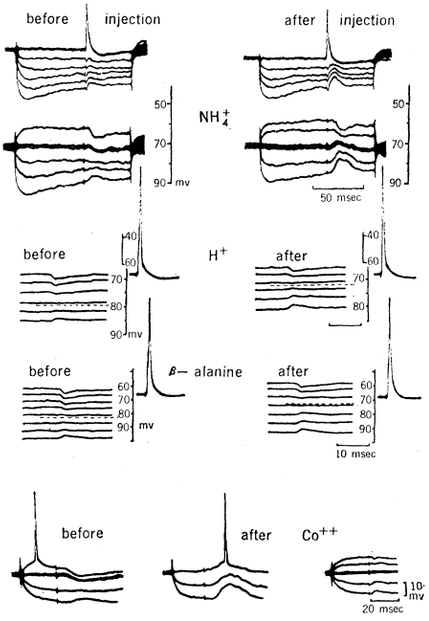

Fig. 1. Shifts of the IPSP's after injection of various ions; IPSP's recorded at different membrane potentials are superimposed. The cell injected with ammonium ions shows antidromic IPSP's above and polysynaptic IPSP's below, after the stimulation artifact. The membrane potential slopes during application of current steps are unchanged. The antidromic action potential (87 mv) elicited at resting potential is truncated. Injection current was 20 na for 1 minute; delay after injection was 35 minutes. The direct group I IPSP's in the cells injected with hydrogen ions (50 na for 2 minutes) and with β-alanine (100 na for 2 minutes) are recorded during prolonged current steps. Delay after injection was 40 minutes. The injection of cobalt ions was 20 na for ½ minute. The equilibrium potential of the group Ia IPSP (to the right) is at resting potential of about 62 mv. The polysynaptic IPSP (to the left) was also hyperpolarizing by about 3 mv before injection. The probable IPSP admixture leads to cell firing after injection (20 minutes later).

To clarify the mechanism leading to this surprising effect on the IPSP, we extended the experiments to related substances. Our results demonstrate that a number of ions with positive charges, particularly ammonium ions, amino acid ions, hydrogen ions, and ions of some heavy metals, act predominantly and with long-lasting effect upon the IPSP-generating mechanism.

Cats (35) were anesthetized with pentobarbital. The results are from 164 motoneurons from L_7 and S_1 segments. We elicited IPSP's by way of hind-limb muscle and skin nerves and by the ventral roots. Three-barreled micropipettes (*1*) were used. We filled two of the barrels with the substances to be applied by iontophoresis. Amino acid solutions were adjusted to a *p*H 6 to 8 by addition of acetic acid or potassium hydroxide. To obtain the necessary charge for iontophoresis of the more neutral amino acids, we prepared both alkaline and acidic solutions from *p*H 2 to 4 and *p*H 7.5 to 11. We tested these amino acids in both solutions in order to discriminate between their action and that of hydrogen and hydroxyl ions. The latter were studied with buffered acetic acid, phosphoric acid (*p*H 2), and potassium hydroxide (*p*H 12) solutions. The solutions of cupric and cobalt acetate and ferrous sulfate were buffered to *p*H 7 to 8.

Hydrogen ions were effective in depolarizing the IPSP but hydroxyl ions were not. An effect on it was observed with lysine, histidine, β-alanine, glycine, and leucine in all except 2 of 102 cells and in all cells with ammonium, cobalt, and cupric ions. Injection of glutamate (*3*), aspartate, and ferrous ions was ineffective in most cells. That the application was intracellular was confirmed autoradiographically by the use of tritiated amino acids in 17 experiments. Excitatory postsynaptic potentials remained unaltered.

Current injection was started after an observation period of 5 to 20 minutes at stable resting potential (Figs 1 and 2). The E_{IPSP} was determined by recording the amplitude and sign of the IPSP at different membrane potentials which were determined by transmembrane current steps. Repeated determinations of its reversal point after the injections revealed a considerable and long-lasting depolarizing shift of the equilibrium potential for IPSP's of recurrent afferent, group Ia,b, and polysynaptic origin. An obvious decrease of this shift was not seen within 10 minutes; restitution was found incomplete even 90 minutes after injection. The shift was independent of the behavior of the resting membrane potential. This usually remained substantially unchanged during low-current iontophoresis but was depolarized up to 20 mv in most cells if high iontophoresis currents had to be applied, sometimes with transient antidromic inexcitability. The depolarization recovered within 4 to 15 minutes, which was long before a restitution of the inhibitory potential.

The iontophoretic currents necessary to elicit a clear reduction of the IPSP's ranged between 8 na for 1 minute and 300 na for 3 minutes. The overall effective range for the injection currents was NH^+_4 > lysine ≧ histidine > β-alanine = glycine ≧ leucine ≫ aspartate, glutamate and for the other ions $Co^{++} > Cu^{++} > H^+ > Fe^{++}$. We found no obvious difference between the injection of ions by comparable currents across the membrane and across the two injection barrels.

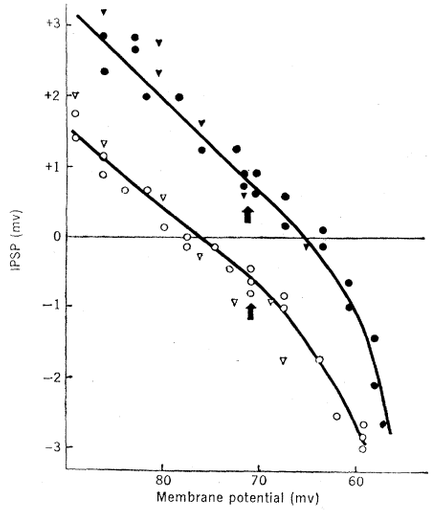

Fig. 2. Size and sign of IPSP is plotted as a function of membrane potential before, and 30 to 40 minutes after, injection of lysine (50 na for 2 minutes). Open circles, group Ia IPSP before injection; open triangles, recurrent IPSP before injection. Closed circles and closed triangles are the same as open symbols, except after injection. Arrows indicate resting potentials.

When the reversal potential of the IPSP had shifted, its "strength," indicated by the slope of the curve expressing the dependence of inhibitory potential on membrane potential, was not diminished, and convergence of the curves was always present at depolarizing membrane potentials (Fig. 2). A shunt of the inhibitory potential by an increased membrane conductivity was only found when the cells were depolarized during the initial period after injection. After the membrane potentials had recovered, determinations of neuronal input resistance and time constant in these cells revealed no significant deviations from the previous values. The maximum shift in membrane potential seen in the motoneurons studied was from 82 to 63 mv. Repeated injections (up to 10 times the effective dosage) did not surpass this limit. We found exceptions in cells which deleteriously depolarized after injection and in which the internal ionic milieu must have been massively altered. These cells were thus excluded.

The postulated limited pore sizes of the postsynaptic inhibitory membrane, as derived from extensive studies (*3*, *4*), should prevent the penetration of most of the ions studied, except for ammonium and hydrogen ions, since their dimensions in the hydrated form are too large. Ammonium ions show such similarity to potassium ions (*5*) that they could hardly be distinguished by the postsynaptic inhibitory membrane pores. The effects of injections of potassium ions or salt are essentially attributed to intracellular accumulation or diminution of chloride (*3*, *4*). Thus, as with potassium ions, one should expect a small and short-term depolarizing E_{IPSP} shift after injection of ammonium in cationic form or a hyperpolarizing shift if ammonium together with the impermeable acetate is applied by cross-barrel injection. Since both forms of application result in similar effects, it seems unlikely that the effect described here can, in general, be derived from intracellular changes of the concentration of chloride ions. There are direct findings which strengthen this supposition. In contrast to the effect of those ions, such as chloride, that participate in the IPSP ionic current flow, the ions studied here caused IPSP equilibrium shifts that were restricted to a rather fixed upper limit of about 60 mv. This shift was observed without a concomitant membrane potential depolarization, which does not favor the assumption that intracellular chloride ion is retained (*3*). Compared to the values reported for other ions (*3*, *4*), the observed times of decay of the shift are of extreme duration. Whether these different times of decay can occur simultaneously was studied in the separate experiments in which chloride ions were also injected. Even if a maximum shift of the equilibrium potential was obtained, the chloride injection that followed resulted in an additional shift. The recovery time from chloride injection was as fast as in normal cells (1 to 2 minutes).

Since chloride ions still pass and the observed limit of the E_{IPSP} shift is comparable to the proposed electrochemical chloride potential (*3*, *4*), the IPSP potassium channel of the treated cells seems to be inhibited and the chloride channel maintained or favored. Both actions would account for the maintained strength of the inhibitory potential as defined above. An action on the postsynaptic inhibitory membrane by saturation of negative charges at a cationic exchange site perhaps offers an explanation of such an alteration of ionic flow. Although the effects are similar for the organic and inorganic ions studied, it remains in question whether the processes underlying their action are identical.

A nerve cell is less likely to undergo inhibition if it shows a reduction of the postsynaptic inhibitory potential at the normal resting membrane potential. It should be noted that such a diminution of inhibition is produced by those substances (ammonium, hydrogen ions) which are increased in epileptogenic states (*6*).

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A Molecular Basis for Heterosis

Abstract. *Alcohol dehydrogenase allodimers composed of an unstable active subunit and a stable but inactive subunit are both active and stable. The implication of this finding for the problem of heterosis is discussed.*

Heterosis, or hybrid vigor, remains an intriguing problem despite the fact that its genetic basis has been under investigation for the greater part of this century. It is very likely a complex phenomenon which can result from many types of gene and allele interactions. Below we present evidence for a form of allele interaction which results in increased enzyme activity in certain heterozygotes and may thus serve as a model for single gene heterosis.

Maize is polymorphic for the alcohol dehydrogenase (E.C.1.1.1.1.) gene, Adh_1. Four alleles have been described (*1*). The Adh_1^S, Adh_1^F, and $Adh_1^{C(t)}$ alleles specify enzymes which differ in charge. The enzyme is a dimer, and three isozymes are formed in heterozygotes. In zymograms of extracts from mature kernels the two autodimer isozyme bands are of equal intensity and the allodimer (hybrid enzyme) is approximately twice as intense. The fourth allele, $Adh_1^{C(m)}$, produces an enzyme which has the same electrophoretic mobility as that specified by $Adh_1^{C(t)}$ but is much less active. The C^mC^m dimer shows only about 1/30 of the activity of the other three autodimer isozymes. An allodimer enzyme composed of the weakly active C^m protomer and an active protomer is about one-half as active as a dimer composed of two active protomers; and the FC^m and FF isozyme bands in $Adh_1^F/Adh_1^{C(m)}$ heterozygotes are of approximately equal intensity. The same condition is observed in the $Adh_1^S/Adh_1^{C(m)}$ heterozygotes.

Studies on the physical-chemical properties of the isozymes specified by the various alleles have revealed that the C^mC^m isozyme differs strikingly from the isozymes specified by the other three alleles in its stability at high *p*H (*2*). The relatively inactive C^mC^m enzyme is very stable at *p*H 10.0, whereas