these experiments. After impalement there was a rapid downward (negative) deflection of the microelectrode potential, which reached a minimum (− 87 mV) about 1 minute after impalement. The delay probably reflects local membrane damage followed by spontaneous repair at the site of impalement (*1, 3, 6*). On reaching its minimum value after impalement, the potential remained steady until the microelectrode was withdrawn 3 minutes after a steady state was established. After withdrawal, the potential returned rapidly to its initial value (7 mV) in the mucosal medium.

The equation $a^{i}_{Na}/a^{o}_{Na} = 2.303 \exp [(\Delta E - E_m)/S_{NaK}]$ was used to calculate a^{i}_{Na} from 14 recordings, similar to Fig. 1B, in tissues from six different animals. Here ΔE is the difference between the steady-state microelectrode potentials in the cell interior and mucosal medium (average value, − 88.2 mV), E_m is the mucosal membrane potential, and S_{NaK} is the slope of the microelectrode used to measure each value of ΔE. The average E_m (− 35 mV) found previously (*6*) in 104 impalements with tissues from 11 different animals was assumed in the present study.

The a^{i}_{Na} in these experiments was 9.1 ± 0.9 m*M*. This agrees reasonably well with the value previously found (*6*) with Na^+-selective microelectrodes containing monensin (6.3 m*M*). We believe, however, that this agreement reflects the capacity of poorly selective microelectrodes, when properly used, to yield reasonably accurate estimates of a^{i}_{Na} under steady-state conditions (*4, 6*), rather than the utility, for this purpose, of the highly Na^+-selective microelectrodes described in this report.

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12. Aside from the Na^+-selective ligand (*9*), this solution is similar in composition to Corning 477317 K^+-exchanger. We found that a solution of the ligand in Corning 477317 can be used to make Na^+-selective microelectrodes similar to those described here.
13. Supported by USPHS grant AM 12715. J. F. G.-D. was partially supported by the Ministerio de Educacion y Ciencia, Madrid.

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Membrane Surface Potential Changes May Alter Drug Interactions: An Example, Acetylcholine and Curare

Abstract. *Curare is known to be less effective as an acetylcholine antagonist when the divalent cation concentration of the extracellular solution is increased. This observation can be accounted for by the negative surface potential on the end plate; an increase in divalent cation concentration decreases the negativity of the surface potential and thereby lowers the concentrations of cations at the membrane-solution interface. The concentration of divalent cations, such as curare, will be reduced more than the concentration of univalent cations, such as acetylcholine. The observations can be accounted for by a surface potential of about −50 millivolts. The same principle can explain the reported actions of divalent cations on the affinity of receptors for acetylcholine. The effects of surface potential on concentrations at active sites may play an important role in drug interactions.*

The potency of drugs that act on the acetylcholine (ACh) receptor at the neuromuscular junction can be modified appreciably by changes in the concentrations of ions in the extracellular solution. Increasing the concentration of either Ca^+ or Mg^{2+} decreases the effectiveness of tubocurarine as an antagonist of ACh or of carbachol-induced depolarizations (*1*). However, the effectiveness of curare is enhanced when the Na^+ concentration in the extracellular solution is reduced (*2*). These observations can be accounted for by the negative surface potential found at the end plate.

Most biological membranes have an excess of fixed, anionic groups at the membrane-solution interface. The membrane potential as measured with an intracellular microelectrode is the total voltage drop across the system. In reality this is the sum of at least three subcomponents: the surface potentials at the outer and inner interfaces and the voltage drop through the membrane itself. Increasing the divalent ion concentration in the extracellular solution reduces the negativity of the external surface potential, by screening or binding to the fixed anionic groups. If the membrane potential is held constant (with a voltage clamp, for example) the decrease in the external surface potential is accompanied by a corresponding increase in the voltage drop within the membrane. Consequently, an increase in the extracellular divalent ion concentration has the same effect on the voltage gradient through the end-plate membrane as a hyperpolarization produced by a voltage clamp (*3*). Both treatments increase the duration of miniature end-plate currents (MEPC's). Mallart and Molgo (*4*) showed that a similar lengthening is produced by lowering the extracellular *p*H. This can be accounted for if the hydro-

Table 1. Estimates of the surface potential of the muscle end plate based on curare-ACh interactions in a variety of experimental solutions. See Jenkinson (*1*) for details of the solutions. The mean ψ_s value is 49.7 ± 14.2 mV (± standard error of mean).

Experimental solution	Method	K_1'/K_1	$\Delta\psi$ (mV)	ψ Control (mV)
1. 5.4 m*M* Ca^{2+}	Microelectrode	0.52	+8.15	−87
2. 8.6 m*M* Mg^{2+}	Microelectrode	0.63	+5.87	−42
3. 5.4 m*M* Ca^{2+}	External recording	0.72	+4.07	−45
4. 2/3 Na^+, 1/3 sucrose	External recording	1.11	−1.28	−7
5. 1/3 Na^+, 2/3 sucrose	External recording	2.41	−11.01	−74
6. 8.6 m*M* Mg^{2+}	External recording	0.62	+6.08	−43

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gen ions neutralize anionic groups on the membrane and thereby reduce the surface charge. A comparison of the time course of MEPC's in solutions with increased divalent cations or decreased *p*H with the time course of MEPC's during hyperpolarization produced by the voltage clamp suggests that the end plate has a surface potential approximately between −50 and −100 mV (*5*).

The external surface potential is related to the pharmacology of the neuromuscular junction because it sets the concentrations of ions close to the membrane, $[X]_s$, compared to their concentrations in the bulk solution, $[X]_b$, according to the Nernst equation:

$$\psi_s = -RT/(zF) \ln \{[X]_s/[X]_b\} \quad (1)$$

where ψ_s is the surface potential, R is the gas constant, F is Faraday's constant, T is absolute temperature, and z is the valence of the ion. The larger the valence, the greater the effect of the surface potential. Acetylcholine is a univalent cation. With a surface potential of −60 mV, ACh will be concentrated tenfold at the membrane surface compared to the bulk solution. The same −60-mV surface potential will concentrate the divalent antagonist tubocurarine 100-fold near the membrane surface. Since the effect of the membrane surface potential is to concentrate the agonist and antagonist to differing extents, changes in the magnitude of the surface potential should alter the apparent affinities of ACh and curare.

The relevant data for quantifying this hypothesis have been published. Jenkinson (*1*) recorded the depolarization of the end-plate region of frog extensor digitorum longus IV muscles in response to ACh, using an air-liquid interface as an extracellular electrode or with an intracellular electrode. The response was recorded with different concentrations of ACh and tubocurarine, and also in solutions with altered concentrations of Ca^{2+} or Mg^{2+}. When both agonist and antagonist bind to the same site on the receptor (competitive inhibition) the affinity of the antagonist, K_i, is given by

$$[A]/[A'] - 1 = K_i[I] \quad (2)$$

where [A] is the concentration of agonist that elicits a given response in the presence of a set concentration of antagonist [I], and [A'] is the concentration of agonist that produces the same response in the absence of antagonist (*6*). The concentrations are those in the bulk solution. If the receptor is on a membrane, and if the membrane has a surface potential, then the effective concentrations at the receptor site are as described by Eq. 1.

Suppose that the membrane surface potential is changed. The concentration of ACh in the bulk solution required to produce the same response will change to an equal extent for both [A] and [A']. In other words, a change in surface potential leaves the left-hand side of Eq. 2 unaltered. Differences in the measured K_i's then reflect the change in antagonist concentration at the membrane produced by the change in surface potential. On these assumptions

$$-RT/(zF) \ln K_i'/K_i = \Delta\psi_s \quad (3)$$

where K_i is the measured affinity constant in the control solution and K_i' is that from the solution with altered cation concentrations. Since the valence of tubocurarine is two, a tenfold change in apparent affinity indicates a 30-mV shift in surface potential.

If the concentration of univalent ions in the extracellular solution is decreased, there will be an increase in the negativity of the surface potential and therefore a relative increase in the concentration of tubocurarine at the receptors and an increase in the calculated affinity constant.

By using the Grahame equation (*7*) along with the change in surface potential calculated with Eq. 3, it is possible to estimate the control membrane surface potential in each experiment. Table 1 summarizes Jenkinson's results and our calculations of the changes in surface potential for each of his experiments. Though there is appreciable scatter, it appears that there is a substantial negative potential at the external membrane surface of the end plate. The average value is about −50 mV, which is lower than our initial estimate of roughly −100 mV from experiments with altered divalent cation concentrations (*3, 5*) but identical to the estimate of Mallart and Molgo (*4*) from experiments with lowered *p*H.

Our calculations involve important assumptions. We assume that the actual affinity of the receptor is unaltered by the experimental treatments. We assume that there is a smeared, uniform charge density on the end plate, and that the changes in univalent and divalent cation concentrations change the potential only by screening. If, as seems likely, the divalent cations bind to negative charges on the membrane, our estimates of surface potential are high. We also assume that the binding of the agonist and antagonist to the receptor does not significantly modify the local charge density.

We suggest that the surface potential on the membrane containing the receptor, and its modification by changes in the ionic composition of the bathing solution, will help to explain many curious observations in the pharmacology of the neuromuscular junction and of other systems as well. For example, the blocking potency of decamethonium, a divalent antagonist, is increased about twofold when the extracellular solution contains 1.8 m*M* Ca^{2+} instead of 10 m*M* Mg^{2+} (*8*). This is readily accounted for by a −50-mV surface potential on the end plate. Some apparent experimental paradoxes may also be resolved. Del Castillo and Engbaek (*9*) reported that (i) Mg^{2+} reduces the depolarization produced by bath-applied ACh and (ii) Mg^{2+} in the presence of curare increases the depolarization produced by bath-applied ACh. The two observations are reasonable as long as there is a significant end-plate surface potential. Mg^{2+} reduces the surface potential and this reduces local ACh concentration (and ACh-induced conductance changes), but Mg^{2+} causes an even greater reduction in the local curare concentration. This effect is dominant and leads to a net increase in the response. There are many observations reported about the effects of changes in the cation concentration of the extracellular fluid on the potency of ACh, which have lead to some elaborate theories about the binding of ACh to the receptor involving an ion exchange reaction (*10*). Clearly these observations should now be reevaluated in view of the surface potential on the end-plate membrane, which provides a simple explanation for many of the experimental results.

In a more general context, the results point out the importance of considering the valences of agonist and antagonist, as well as the surface potential of the membrane, in estimating the affinity of drugs for their receptor sites. Similarly, alteration of the valence of agonists and antagonists should provide a means of modulating apparent affinities of drugs for receptor sites without changing their concentrations in the bulk solution.

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be high because of complications caused by ACh rebinding. The experiments without neostigmine give values between −60 and −70 mV.

6. It seems possible that two ACh molecules bind to the receptor. In this case, Eq. 2 will be altered to $[A]^2/[A']^2 - 1 = K_1[I]$, and Eq. 3 will be unaltered. The value of K_1 obtained from Eq. 2 will be larger and this will give a different estimate for the surface potentials. Nevertheless, all the experiments in Table 1 still yield negative surface potentials if the revised Eq. 2 is correct.
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$$\sigma^2 = \frac{\epsilon kT}{2\pi}[i^+]$$
$$[\exp(Q\psi kT) + \exp(-Q\psi kT) - 2] +$$
$$[i^{2+}][2\exp(Q\psi kT) + \exp(-2Q\psi kT) - 3]$$

where σ is the membrane surface charge density (charges per square centimeter), ψ is the surface potential (millivolts), $[i^+]$ and $[i^{2+}]$ are the concentrations of univalent and divalent ions (moles per cubic centimeter), k is the Boltzmann constant, T is temperature in degrees Kelvin, Q is the charge on the electron, and ϵ is the permittivity of water.

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Lateral Geniculate Nucleus in Dark-Reared Cats: Loss of Y Cells Without Changes in Cell Size

Abstract. *In cats reared in the dark from birth until 4 months of age, the dorsal lateral geniculate nucleus contained few normal Y cells in either the binocular or monocular segments. Although most of the neurons appeared to be normal X cells unaffected by light deprivation, many cells with abnormal receptive field and response characteristics were encountered. These effects were permanent, since 1 to 2 years of normal visual experience following initial light deprivation did not lead to any functional recovery. The sizes of cell bodies in cats reared in the dark were similar to those of normal animals, an indication that changes in geniculate cell physiology need not be related to changes in cell size.*

The cat's retino-geniculo-cortical pathways have been subdivided on the basis of electrophysiological criteria into at least two subsystems. These subsystems, called the X and Y pathways, are composed of retinal ganglion X and Y cells that project to geniculate X and Y cells (*1*-*3*). These neurons in turn project to the visual cortex. Compared with X cells, Y cells generally (i) have axons that conduct more rapidly, (ii) respond to more rapidly moving visual stimuli, (iii) are more phasic in their response to standing contrast, (iv) have larger receptive fields, and (v) display less linear response summation to visual stimuli.

During postnatal development, Y cells in the dorsal lateral geniculate nucleus are more susceptible to visual deprivation than are geniculate X cells. For instance, if the lids of one or both eyes of a kitten are sutured shut just after birth and maintained in this manner for several months, there is a significant reduction in the percentage of geniculate Y cells encountered with microelectrodes in the laminae receiving input from the sutured eye or eyes (*4*-*6*). In contrast, the development of Y cells in the retina appears to be unaffected by lid suture (*7*). Although eyelid suture essentially eliminates patterned visual input, the amount of light striking the retina is reduced during development by only 1 to 2 log units (*8*). To determine the effects of total light deprivation on the development of geniculate X and Y cells, we raised four cats from birth to 16 to 18 weeks in complete darkness. No light source of any sort including infrared was introduced into the rearing area during this period.

At the end of the dark-rearing period, single geniculate neurons were recorded extracellularly in two of the kittens (DR cats), and the two remaining animals were given 1 to 2 years of normal visual experience prior to recording (DR-LR cats). We used tungsten microelectrodes to isolate and study 156 single units in laminae A and A1 of the four dark-reared cats. Electrodes were placed near the rostro-caudal middle of the nucleus, and thus all receptive fields were within a few degrees of the horizontal zero parallel. Details of recording and criteria used for classifying cells as X or Y have been reported (*1*, *2*, *4*, *7*).

All cells encountered in the binocular segment of laminae A and A1 of normal adult cats can be classified as X or Y cells, and they are sampled with roughly the same frequency (Fig. 1A) (*9*). By contrast, in the A laminae of DR and DR-LR cats, X cells comprised the vast majority of the total (Fig. 1A). Only a few of the neurons could be classified as normal Y cells, and about a fifth of the cells had abnormal response properties and could not be classified as normal X or Y cells (*10*, *11*). No difference was found between animals dark-reared with or without additional normal visual experience. Furthermore, no significant interanimal variability was observed among the four animals of our experiment, since the range in percentage of Y cells was 0 to 7 percent.

The ratio of X and Y cells encountered in the A laminae depends on the eccentricity from the vertical meridian of the receptive fields sampled, which in turn relates to the medio-lateral location of the electrode within the lateral geniculate nucleus (Fig. 1B) (*4*). The effects of monocular and binocular eyelid suture differ not only in the ratio of X and Y cells encountered, but also in these ratios as a function of eccentricity (*4*). Cats with monocular lid suture have few Y cells throughout the deprived, binocular segment of the nucleus, yet have a normal ratio of Y cells in the deprived, monocular segment. Such evidence suggests that the Y cell loss observed in cats with monocular lid suture results from an imbalance in visual input between the two eyes (binocular competition) and not from deprivation per se (*4*, *12*). In comparison with normal animals, cats with binocular lid suture suffer a reduction of Y cells throughout the nucleus, including the monocular segment. A similar but more pronounced effect is seen in DR and DR-LR animals (Fig. 1B). In these animals, we found a reduced percentage of Y cells throughout the nucleus. Moreover, we also found that the percentage of Y cells in cats reared in the dark was reduced in comparison with cats with binocular lid suture ($P < .01$, χ^2 test). In fact, the percentage of Y cells encountered in the binocular segment of animals reared in the dark is essentially as low as that reported for the binocular segment of monocularly deprived cats. However, the monocular segment of cats with monocular lid suture differs from that of cats reared in the dark (Fig. 1B).

In addition to the electrophysiological experiments, we also measured the cross-sectional areas of 100 cells in each DR and DR-LR animal. These samples were taken from a zone in the binocular segment of lamina A near the rostro-caudal middle of the nucleus (typically from sections just rostral or caudal to those containing electrode tracks), and only cells with nucleoli in the plane of the section were measured. Medio-laterally, the sample zone was in that area of lamina A which represents the

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