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Letter to the Editor

Neurotransmitter Discharge and Postsynaptic Rise Times

A recent paper by Van der Kloot (1995) contained considerable criticism of evidence from Khanin, Parnas, and Segel (1994) that discharge of a transmitter from synaptic vesicles into the synaptic cleft (following the opening of the vesicles) cannot be governed by diffusion. From now on we will refer to these authors as VdK and KPS, respectively.

The main reasons that led KPS to their conclusion were the following. 1) By diffusion, the duration of discharge will be at least 600 μs (and probably considerably more), a duration that either outlasts the minimal latency in transmitter release or accounts for most of it. 2) In view of the previous reason, discharge, if governed by diffusion, is the slowest step in the chain of events leading to exocytosis (in nerve terminals) and as such must account for the high Q_{10} (3–4), which characterizes the minimal latency. However, diffusion exhibits a low Q_{10}. 3) Based on reason 1, if diffusion governs discharge, the concentration of neurotransmitter expected to reach the postsynaptic area where the receptors are concentrated would be much too low in comparison with the mM range assessed to be there. To obtain this range, the discharge duration must be $\sim$100 μs, irrespective of the mechanism underlying discharge.

According to VdK, KPS assert that diffusion is too slow to account for earlier theoretical estimates, noted in the literature, of rise times of 100-μs; for miniature and plate currents (mepc) but VdK's new experiments and others show that the rise time is twice as long. In fact, KPS never mentioned rise time, and we show here that rise time is indeed a poor prognosticator of what interested them, the duration of discharge. If rise time is nonetheless considered, we use up-to-date parameter values to show that theory and experiments concerning rise time are in accord if discharge lasts 100 μs.

Based on a model described in its caption, Fig. 1 shows two simulated mepcs, one obtained with 100-μs discharge (*A*) and the other with 600-μs discharge (*B*). The rise time (10–90%) in *A* is 165 μs whereas in *B* it is 480 μs (see *inset* and Fig. 2). The experimental rise time for adult mice, from which the rate constants were taken, is 170 $\pm$ 25 μs (eight trials, 20°C, mouse diaphragm; J. Dudel, unpublished results). The agreement with 165 μs (100-μs discharge) is excellent. Even a 50-μs discharge gives a rise time within the experimental range (Fig. 2).

To obtain a rise time of 200 μs, the maximum value that is consistent with the measurements, a discharge duration of $\sim$200 μs is required. Such a duration of discharge is still much faster than the duration that is predicted by diffusion.

Taking a more general point of view, we stress that there is no consistent relation between the rise time and the discharge time, for the former is longer than the latter for brief discharges and shorter for longer discharges (Fig. 2). The discrepancy is more pronounced for the 20–80% criterion used by VdK. We also note the insensitivity of the rise time to changes in the discharge time; we have seen that doubling the discharge time from 100 μs to 200 μs results in only a 20% increase in the rise time.

Three further points remain to be made.

1. VdK used the same simplified equation as KPS to describe diffusion through the fusion pore. However, KPS applied this equation to calculate concentration in the vesicle, whereas VdK used it to calculate concentration in the synaptic gap. The latter application requires approximating the gap volume by the vesicle volume, a major underestimate. This leads to significant overestimates of gap concentration.
2. VdK attacks the use of temperature sensitivity by KPS, stating that this sensitivity arises mainly from protein-assisted vesicle opening; he asks what this has to do with the rise time. The question is irrelevant, because KPS considered the temperature sensitivity of something different, the synaptic delay. And KPS considered this delay precisely because, unlike rise time, synaptic delay does provide good diagnostic information about the brief discharge process.
3. VdK dismissed the idea of ion-exchange as the underlying mechanism for discharge, as was suggested in the Discussion of KPS and has since been elaborated (Khanin et al., submitted for publication). This mechanism relies on the exchange of a charged transmitter with an external co-charged ion. For positively charged acetylcholine, for example, a suitable positive ion is Na^+, the most prevalent extracellular positive ion. Obviously Ca^{2+} and any other positive ion in the medium can also take part in the exchange. In fact, for a bivalent ion, a lower concentration is needed to exchange for the content of the vesicle. Therefore, it is not surprising, and in fact is even encouraging, that Katz and Miledi (1969) recorded miniature end plate potentials in isotonic $CaCl_2$ solution. This certainly cannot be taken, as suggested by VdK, as "evidence that Na^+ does not play a special role in ACh release."

To summarize, as did KPS indirectly, the new calculations presented here directly support the assertion of VdK's title, that "the rise times of mepc suggest that acetylcholine may be released over a period of time," and they give a quantitative estimate of that time. But the considerations raised by VdK do not weaken the case made by KPS that diffusion is too slow to account for synaptic transmitter

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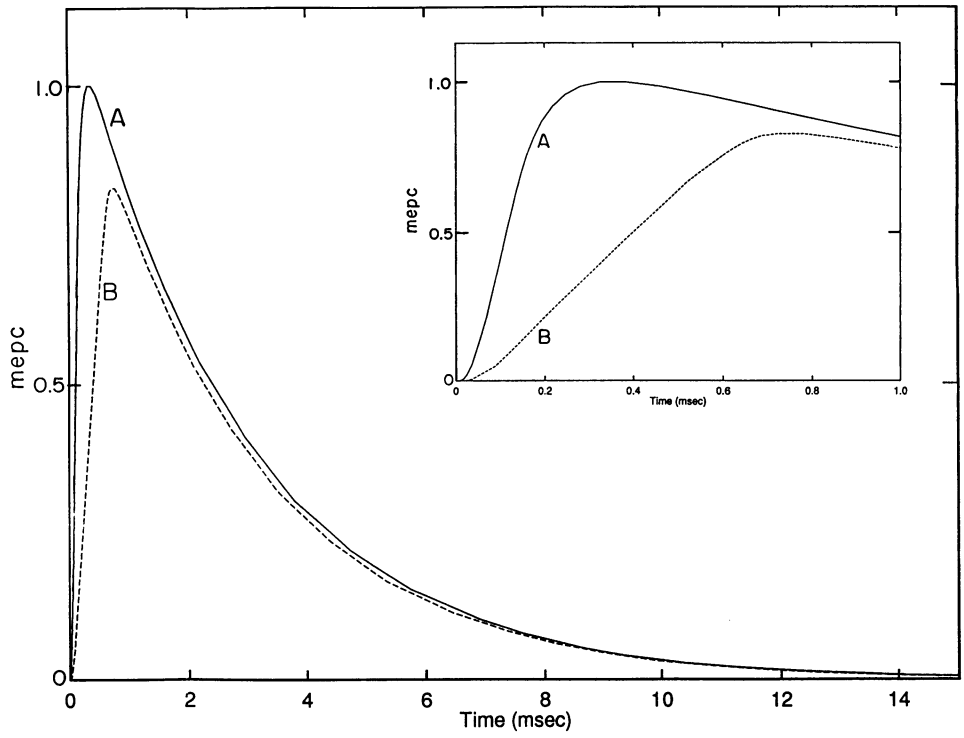

FIGURE 1 Calculated miniature end plate currents (mepc, normalized) with discharges, respectively, of 100 (*A*), and 600 (*B*) μs. *Inset*: the first ms, enlarged. The processes in the synaptic gap were modeled as follows, using the FIDAP software package (version 7.5, FDI inc., Engelman, 1991) ACh is released from the center of a release site through a fusion pore of 1-nm radius during a chosen time (modeled as a step current of ACh with a total charge of 10^4 molecules). Then ACh diffuses through the synaptic gap to the postsynaptic membrane, where the receptors are placed. The hydrolysis of ACh by ACh-esterase was modeled as a 2-stage enzymatic reaction according to Parnas et al. (1989). The ACh receptors were modeled according to Franke et al. (1991) (except for the closing rate, which was taken to be 850 s^{-1} instead of 1100 s^{-1}). The conductance change, especially during the rise time, is brief. Thus we took the mepc to be proportional to the calculated number of open channels. The missing rate constants (related to receptor desensitization and resensitization) were taken from Bufler et al. (1995). The total amounts of ACh esterase and ACh receptors were taken from Anglister et al. (1994). The diffusion coefficient of ACh in the synaptic gap was set at $2 \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$. With these parameters, an excellent fit was obtained to relevant results of J. Dudel in mouse (unpublished).

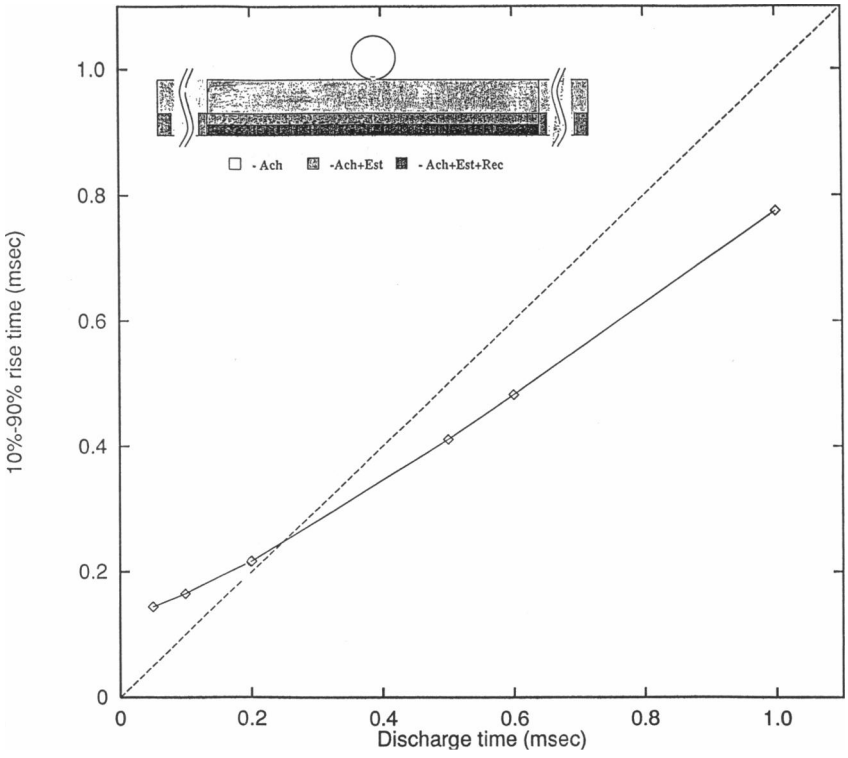

FIGURE 2 Calculated rise time of mepc vs. vesicle discharge time (*solid line*). *Dashed line*: Expected graph if the two times were equal. *Inset*: the geometric layout used in the computer simulations. Circle represents vesicle of 50-nm diameter. The lateral extent of the synaptic gap was taken to be 3000 nm. Ach: acetylcholine. Est: acetylcholinesterase. Rec: receptors (height of receptor zone: 3.76 nm).

discharge and that ion exchange might account for the discharge; in fact they strengthen the case.

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Response to Khanin et al.

I am indebted to Khanin, Parnas, and Segel for stirring my interest in MEPC rise times, so I regret our continuing differences about interpretations. The disagreements are laid out in the two papers (Kahin et al., 1994; Van der Kloot, 1995) and in their letter for all to judge. Two points should be reiterated, because they clearly show that we are not communicating.

In regard to their hypothesis that the exchange of a counterion is required for acetylcholine release, they proposed that the counterion is Na^+. In passing, I noted that Katz and Miledi (1969) had recorded MEPCs in isotonic $CaCl_2$ solution, showing that Na^+ does not play a special role in release. However, the data presented in my paper showed that rise times were about the same as usual when the MEPCs were recorded in isotonic sucrose solution, in which the concentrations of potential counterions must be greatly reduced (see also Miledi et al., 1980). They respond to the passing comment but not to the data.

They continue to make much of the high Q_{10} for the synaptic delay, which is the latent period between the nerve terminal action potential and the beginning of the endplate current (Katz and Miledi, 1964). The time required for the acetylcholine to diffuse across the cleft is very short, so the delay is largely occupied by the steps occurring before release is initiated. I remain confused about how this delay, or its temperature sensitivity, provides (as stated in their letter) "... good diagnostic information about the brief discharge process," as it occurs before discharge begins.

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