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On the role of the baseline firing rate in determining the responsiveness of cingulate cortical neurons to iontophoretically applied substance P and acetylcholine

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The relationship between baseline firing rate and the magnitude of excitatory responses to iontophoretically applied substance P (SP) and acetylcholine (ACh) was determined for neurons in the anterior cingulate cortex of the rat. Whereas the size of responses to ACh was positively correlated with the level of ongoing neuronal activity, no correlation, either positive or negative, could be demonstrated for responses to SP. It seems unlikely that the excitatory effects of SP in this brain area result from release of endogenous ACh.

Neurons in the deeper layers of the cerebral cortex of both cat and rat are strongly excited by the iontophoretic application of acetylcholine (ACh) (Crawford & Curtis 1966; Krnjevic et al 1971; Stone 1972; Lamour et al 1982). In the cat, Crawford & Curtis (1966) reported that cells with 'moderate to high' levels of spontaneous firing were more likely to be excited by ACh. Also, Krnjevic et al observed that on cat cortical cells the magnitude of the excitation evoked by ACh tended to vary directly with the baseline firing rate. In the present study we determined if there was any relationship between cell firing rate and size of response to ACh for cells in the cortex of the rat.

Iontophoretic application of substance P (SP) to deeper neurons in the rat cortex also evokes pronounced and prolonged excitatory response (Phillis & Limacher 1974; Jones & Olpe 1982; Lamour et al 1983). Since SP has been shown to cause release of ACh from cerebral cortex (Pepeu 1974), the coincidence of neurons excited by SP and ACh has led some authors to consider that SP-evoked excitations are not direct but due to release of endogenous ACh (Phillis & Limacher 1974; Lamour et al 1983). This possibility seems to be strongly denied by the observation that in the cortex (Phillis & Limacher 1974; Lamour et al 1983) and other brain regions (Sastry 1978; Guyenet & Aghajanian 1979) SP-evoked excitations are not blocked by cholinergic antagonists. Nevertheless, Lamour et al (1983) have proposed that SP and ACh, although acting on different receptors, may share a common postsynaptic mechanism of action. In view of this we have also determined whether the baseline firing rate plays any role in determining cortical neuron responsiveness to SP.

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Methods

The experiments were performed on male albino rats (RAIf, 200–400 g) anaesthetized with chloral hydrate (400 mg kg^{-1} i.p.). Conventional techniques were used to record action potentials extracellularly from single neurons and to apply substances by iontophoresis using 4-barrelled microelectrodes. Two barrels of each electrode contained NaCl (4 M), one for recording action potentials and the other for continuous balancing of the iontophoretic currents. The remaining barrels contained SP (0·001 M dissolved in 0·165 M NaCl, pH 5) and ACh Cl (0·1 M, pH 6).

All the cells studied were spontaneously active and were located between 750 and 1200 μM from the pial surface in the anterior cingulate cortex (6–7 mm anterior to the interaural line, 0·5–1·0 mm lateral to midline). This particular cortical area was studied for several reasons. It has a clear, albeit sparse distribution of SP-containing nerve terminals (Inagaki et al 1982) and a moderate density of SP-binding sites demonstrable by autoradiography (Quirion et al 1983). The neurons in the rat cingulate cortex are excited by SP and its fragment peptides with a similar structure activity profile to that seen in other systems (Jones & Olpe 1982). Finally, in man, at least, the anterior cingulate cortex has a higher SP-content than any other cortical area (Crystal & Davies 1982).

Standard applications of SP (80 nA, 60 s) and ACh (30 nA, 20 s) were used to evoke submaximal excitatory responses on cingulate cortical cells. The responses were quantified by measuring the total number of action potentials produced over and above the baseline firing rate, in response to the agonist application. This was termed the spike number. Baseline firing rate was measured as spikes per second and related to response size using a two-variable regression analysis.

Results

The percentages of cells excited by SP and ACh in the limited region studied (between 750 and 1200 μm) were very high. About 84% of cells were excited by ACh and the percentage responding to SP was even higher (93%).

The relationships between spike number and firing rate for SP and ACh are shown in Fig. 1. With SP, no significant correlation between the two variables could be demonstrated. Thus, it seems that responsiveness to

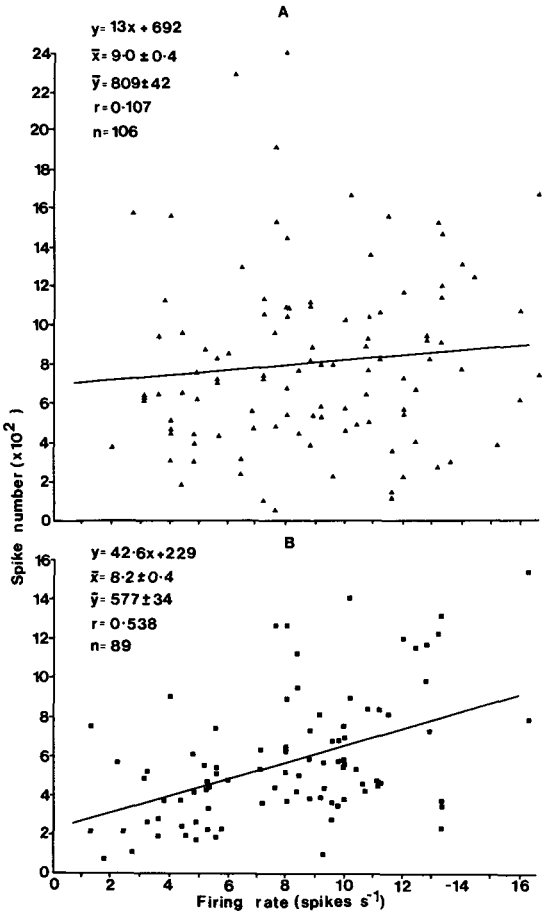

FIG. 1. The plot in A shows the relationship between response size and baseline firing rate for cells excited by substance P. No significant correlation in either a positive or negative direction could be demonstrated. In contrast, the two parameters showed a significant positive correlation on cells excited by acetylcholine (B).

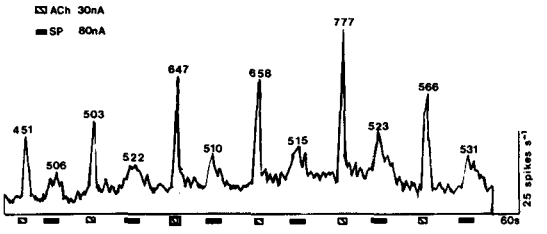

FIG. 2. The trace is a ratemeter record from a single cingulate cortical neuron excited by alternate applications of acetylcholine and substance P. The numbers above the responses are the number of action potentials evoked over and above the baseline firing rate. It can be seen that a gradual rise in baseline is accompanied by an increase in the response to acetylcholine and the drop in firing towards the end of the trace is concurrent with a return of the cholinergic response towards control levels. In contrast, the responses to substance P remain constant throughout.

SP is independent of the ongoing activity of the neuron. In contrast, however, there was a significant positive correlation between spike number and firing rate demonstrable for ACh indicating that the more active cells were more sensitive to the application of exogenous ACh.

On a number of cells it was possible to observe the effects of spontaneous shifts in the baseline firing rate on the responses to the iontophoretically applied agonists. In general, the size of responses to SP appeared to be insensitive to alterations, either up or down, in baseline firing rate but ACh responses tended to vary directly with the baseline changes. One good example is shown in Fig. 2 where responses to the two substances were alternated on the same cell. A gradual rise in the baseline firing rate was associated with increased responses to ACh but little change in SP-evoked responses.

Discussion

Krnjevic et al (1971) predicted from intracellular studies on cat cortical neurons that the excitatory effects of ACh would vary directly with the level of spontaneous activity. The present study confirms this suggestion on rat cortical neurons since the size of responses to ACh was directly correlated with the spontaneous firing rate. It seems logical that such correlation would exist. The cells with higher firing rates are presumably more depolarized and hence more excitable than those with lower spontaneous activity and thus might be expected to be more responsive to the exogenous application of ACh. It could also be speculated that the faster firing cells may have a greater density of cholinergic receptors and thus be under a greater influence from a tonically active cholinergic input. The latter almost certainly is operative in the cortex. Spontaneous release of ACh from cortex is demonstrable in-vivo (Conte et al 1982) and iontophoretically applied atropine or cholinesterase inhibitors can decrease or increase cortical cell firing respectively (Spehlmann 1963; Stone 1972; Lamour et al 1982). Thus the spontaneous firing of cortical cells is probably maintained, at least in part, by the cholinergic input. The differences in firing rates and hence response size could be partly a function of the density of neuronal cholinergic receptors therefore.

The failure to demonstrate any relationship between firing rate and size of SP-responses seems surprising. If it is assumed that the excitatory effects of SP result from a direct postsynaptic depolarization, then it might be expected that the more active, and presumably more excitable cells would exhibit greater responsiveness to the peptide. This failure seems even more surprising when one considers the similarity between the probable ionic mechnism underlying SP- and ACh-evoked depolarization. Intracellular studies have consistently shown that the muscarinic-depolarization of neurons by ACh is accompanied by an increase in membrane resistance, probably reflecting a decrease in K^+-conductance

(Krnjevic et al 1971; Dodd et al 1981) and similar observations have been made with regard to the action of SP (Krnjevic 1977; Nowak & MacDonald 1981; Ogata & Abe 1982). It seems likely that the K^+-current involved in the action of ACh is the M-current first observed in the bullfrog sympathetic ganglion (Brown & Adams 1980) and since described for mammalian central neurons (Halliwell & Adams 1982). Recently, SP has also been shown to inhibit this current in frog sympathetic neurons (Adams et al 1983). In view of this close similarity in the likely mechanism of action of the two agents, it is difficult to understand why the responses to one, but not the other, vary with baseline firing.

One possibility which should be considered is that the responses to SP are not simply the result of direct postsynaptic effects of the peptide. As well as clearly documented direct depolarizing actions on neurons (Nowak & MacDonald 1981; Ogata & Abe 1982) SP has also been shown to have pre-synaptic facilitatory and inhibitory actions at synaptic junctions where ACh is the presumed transmitter (Steinacker & Highstein 1976; Steinacker 1977). Also, there are reports that SP can inhibit (Steinacker & Highstein 1976; Krnjevic & Lekic 1977; Lamour et al 1983) and potentiate (Krivoy 1983) the postsynaptic actions of ACh as well as indications that the peptide can reduce neuronal responses to GABA and glutamate (Barker et al 1980). It seems quite likely then that the responses of cortical neurons to SP may be the ultimate manifestation of a number of simultaneous different (even conflicting) actions of the peptide. If this is so then it may not be so surprising that there is no demonstrable correlation between responsiveness to SP and the level of excitability of the neuron.

With regard to the possible interactions of SP and ACh described above, two substances have recently been shown to co-exist in a pathway projecting from the pontine tegmentum to, amongst other areas, the cerebral cortex (Vincent et al 1983). This raises the possibility that SP may act as an endogenous modifier of cholinergic transmission in the cortex or vice versa.

REFERENCES

Adams, P. R., Brown, D. A., Jones, S. W. (1983) Br. J. Pharmacol. 79: 330–333

Barker, J. L., Gruol, D. L., Juang, L.-Y. M., MacDonald, J. F., Smith, T. G. (1980) Neuropeptides 1: 63–82

Brown, D. A., Adams, P. R. (1980) Nature (Lond) 242: 673–676

Conte, G. L., Bartolini, L., Casamenti, F., Marconcini-Pepeu, I., Pepeu, G. (1982) Pharmacol. Biochem. Behav. 17: 933–937

Crawford, J. M., Curtis, D. R. (1966) J. Physiol. (Lond) 186: 121–138

Crystal, H. A., Davies, P. (1982) J. Neurochem. 8: 1781–1784

Dodd, J., Dingledine, R., Kelly, J. S. (1981) Brain Res. 207: 109–127

Guyenet, P., Aghajanian, G. K. (1979) Europ. J. Pharmacol. 53: 319–328

Halliwell, J. V., Adams, P. R. (1982) Brain Res. 250: 71–92

Inagaki, S., Sakanaka, M., Shiosaka, S., Senba, E., Takatsuki, K., Takagi, H., Kawai, Y., Minagawa, H., Tohyama, M. (1982) Neuroscience 7: 251–277

Jones, R. S. G., Olpe, H.-R. (1982) Neuroscience Lett. 33: 67–71

Krivoy, W. (1983) in: Skrabanek, P., Powell, D. (eds) Substance P-Dublin 1983. Dublin, Boole Press, pp 233–234

Krnjevic, K. (1977) in: Von Euler, U., Pernow, B. (eds) Substance P. New York, Raven Press, pp 217–230

Krnjevic, K., Lekic, D. (1977) Can. J. Phys. Pharm. 55: 958–961

Krnjevic, K., Pumain, R., Renaud, L. (1971) J. Physiol. Lond. 215: 247–268

Lamour, Y., Dutar, P., Jobert, A. (1982) Neuroscience 7: 1483–1494

Lamour, L., Dutar, P., Jobert, A. (1983) Ibid. 10: 107–117

Nowak, L. M., MacDonald, R. L. (1981) Ibid. 214: 416–423

Ogata, N., Abe, H. (1982) Neuropharmacology 21: 187–189

Pepeu, G. (1974) Prog. Neurobiol. 2: 257–288

Phillis, J. W. & Limacher, J. J. (1974) Brain Res. 69: 158–163

Quirion, R., Shults, C. W., Moody, T. W., Pert, C. B., Chase, T. N., O'Donohue, T. L. (1983) Nature 303: 714–716

Sastry, B. R. (1978) Brain Res. 144; 404–410

Spehlmann, R. (1963) J. Neurophysiol. 26: 127–139

Steinacker, A. (1977) Nature (Lond.) 267: 268–270

Steinacker, A., Highstein, S. M. (1976) Brain Res. 114: 128–133

Stone, T. W. (1972) J. Physiol. Lond. 225: 485–499

Vincent, S. R., Satoh, K., Armstrong, D. M., Fibiger, H. C. (1983) Nature (Lond.) 306: 688–691