Chapter IV

Discussion and Conclusion

The use of electrophysiological techniques to study actions of DCR on single neurones is distinctly advantageous. The techniques measure directly the electrical activity of a single neurone which is important functional parameter of the nervous system. A convenient method to look for DCR sensitive neurones seems to be to follow single neurone discharges, under the influence of microiontophoretically ejected DCR from a multibarrel microelectrode. In the present study, alkaloid dioscorine is applied microiontophoretically to the Purkinje neurone of the cerebellar cortex which is chosen because of various functional distribution of receptors and neurotransmitters.

DCR produced two distinct effects on cerebellar Purkinje cells. First, it induced excitation as indicated by increased firing rates in a dose dependent manner (Figure 6). Second, it abolished depressant effects caused by microiontophoretic applications of all putative inhibitory neurotransmitters tested, namely, GABA, glycine, taurine, noradrenaline, and 5–hydroxytryptamine (Figure 7–10). By contrast, DCR did not show any discernible effects on excitation induced by application of both glutamate and aspartate (Figure 11–12).

The results from the present study cannot elucidate whether the observed excitation is a direct excitant effect of DCR on Purkinje cell as result of a specific receptor related stimulation. On the other hand, the findings that DCR abolishes depressant actions of all inhibitory putative neurotransmitters tested raises a possibility that the observed excitation may be secondary result of cancellation by DCR of the effects of endogenous inhibitory neurotransmitters spontaneously released onto Purkinje cell. Thus, evidence from other studies indicated that all putative neurotransmitter substances tested in this study, namely, GABA, glycine, taurine, noradrenaline, and 5–hydroxytryptamine, are found in neuronal components surrounding Purkinje cell, and might serve as neurotransmitters in this neuronal tissue (Obata et al., 1967; Otsuka et al., 1971; Ribak et al., 1978; Hokfelt and Ljungdahl, 1972; Tebecis, 1974). Release of these substances in cerebellar cortical tissue has also been demonstrated(Nadi, McBride, and Aprison, 1977; Kawamura and Provinin, 1970; Okamoto et al., 1976; Frederickson et al., 1978; Bloom et al., 1972). Moreover, all these substances exert inhibitory effect on Purkinje cell (Nadi, McBride, and Aprison, 1977; Kawamura and Provinin, 1970; Okamoto et al., 1976; Frederickson et al., 1978; Bloom et al., 1972).

The antagonistic action of DCR on inhibitory neurotransmitters is consistent with behavioural observation in other studies. Thus, DCR produces convulsion in conscious animal (Bhovadhi, 1979; Tantisira et al., 1979). Additional studies also indicate analeptic action of DCR, which

might suggest its therapeutic value (Anothayanontha, 1979; Bhovadhi, 1979; Tantisira et al., 1979; Hokierti, 1980; Tantisira et al., 1984). Antagonism of inhibitory neurotransmitters found in this study could be concluded as mechanism of analeptic actions observed in behavioral studies.

In contrast to BMC and strychnine, which are specific antagonists of GABA and glycine respectively, DCR blocks all depressants tested indiscriminately. The findings that this alkaloid antagonizes all neurotransmitter substances in this study indicate a non–competitive nature of its action. This means that DCR does not act as specific ligand of receptor molecules of any neurotransmitters tested. In fact, it may also be reasonable to postulate that DCR may not act via binding to neurotransmitter receptor molecules.

In attempt to explain the mechanism of DCR action, possibility arises that this alkaloid may act on a specific molecular process which exists as a common mechanism shared by all these inhibitory neurotransmitters. Clearly, receptor mechanisms of these neurotransmitters should be different, as differentiated by specific agonist–antagonist studies for each individual neurotransmitter, e.g. BMC as specific antagonist for GABA (Olsen et al., 1975), and strychnine of glycine (Curtis et al., 1971).

Subsequent to transmitter–receptor interaction there exists ion channel mechanism which occurs as precedence of membrane hyperpolarization which is a common phenomenon observed with most inhibitory neurotransmitters. Typically, mechanisms underlining actions of most inhibitory neurotransmitters involve two ion species, namely, potassium and chloride (Snyder, 1984; Hille, 1992). Thus, transmitter–receptor interaction which causes opening of potassium channels permits efflux of potassium ion and, subsequently, membrane hyperpolarization (Segal, 1981; Nicoll, 1988; North, 1989; Nicoll, Malenka, and Kauer, 1990). Likewise, opening of chloride channels, which, in this case, causes chloride influx, produces the same transmembrane potential change (Bormann, 1988; Sieghart, 1992). In view of these explanations,possibility arises that DCR may act as a blocker of either potassium or chloride channels, or both. Whether this is the case, results from the present study cannot yet be totally supportive, nor can they suggest competitive nature of the proposed ionic antagonism. Nevertheless, further findings in this study that DCR did not block excitatory effects of both glutamate and aspartate at least suggested that this alkaloid did not posses antagonistic effect on sodium and / or calcium channels mechanisms which are common to the actions of both excitants (Armstrong, 1981; Watkins and Evans, 1981; Mayer and Westbrook, 1987; Unwin, 1989).

In order to clarify the proposed possibility, detailed studies which may provide direct evidence, such as the use of patch–clamping and single channel analysis, should be further encouraged.

In conclusion, the present study elucidates in part the mechanism of action of DCR, a convulsant alkaloid extracted from *Dioscorea hispida.* Microiontophoretic application of DCR abolishes depressant actions of all putative inhibitory neurotransmitters substances tested. This antagonism is not likely to mediate through receptor mechanism of these substances. Rather, blockade of potassium or chloride channels, or both, but not sodium or calcium channels, has been postulated as a possible mechanism.