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Variations in Response of the GABA-Picrotoxin-Benzodiazepine Receptor Complex to Flurazepam

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SIMMONDS, M. A. *Variations in response of the GABA-picrotoxin-benzodiazepine receptor complex to flurazepam.* PHARMACOL BIOCHEM BEHAV **18**(2) 299–301, 1983.—Two effects of flurazepam have been studied on electrophysiological responses to muscimol in a slice preparation of rat cuneate nucleus. Flurazepam potentiated the responses to muscimol and also reduced the antagonism of these responses by picrotoxin. Repeated series of experiments over a 3 year period showed significant variations in the potentiation of muscimol by flurazepam which were negatively correlated with the apparent reduction in picrotoxin potency. A more reproducible reduction in picrotoxin potency by flurazepam was obtained when the data were re-calculated to take account of the possibility that picrotoxin might antagonize the potentiating effect of flurazepam as well as the direct response to muscimol. The simplest explanation of this relationship is that flurazepam and picrotoxin mutually antagonize each others effects on the GABA system. The underlying cause of the variation in flurazepam effect remains unknown.

Benzodiazepine GABA receptor Muscimol Picrotoxin Flurazepam

DESPITE the large volume of information on the binding of benzodiazepines to neuronal membranes and the effects of drugs on this binding, it remains important that the actions which benzodiazepines have on functioning physiological systems should also be studied. The classical approach to drug receptor studies on a responding system would ideally require identification of a response to the benzodiazepines which is independent of other transmitter systems. This has not been achieved so far and it does present some difficulties. The only system studied in any detail at the electrophysiological level is the GABA receptor-chloride ionophore complex with its associated regulatory sites for the benzodiazepines and picrotoxin. It is evident that benzodiazepines can potentiate the effects of GABA and its analogues [1–4, 6, 8, 9], albeit modestly, and they can also reduce the potency of picrotoxin but not bicuculline as GABA antagonists [6,8]. In some respects, these effects of benzodiazepines have proved to be rather variable and, in this paper, I shall show how the variation in potentiation of the GABA analogue muscimol is related to the variation in reduction of picrotoxin potency.

The preparation used for this work was a slice of Wistar rat cuneate nucleus in which activation of the GABA receptor complex on the terminations of the dorsal funiculus gave rise to depolarization of the dorsal funiculus fibres [5,7]. All drugs were superfused.

Dose-response lines were obtained to muscimol and parallel displacements of these lines by flurazepam and picrotoxin were measured. The potentiation of muscimol by 10^{-6} M flurazepam was apparent as a left shift of the muscimol dose-response line. The extent of the potentiation was never very large, compared with that induced by pentobarbitone [8] and in any one series of experiments the variation did not appear excessive. With repeated series of experiments over 3 years, however, some significant variation between the series occurred (Fig. 1). It is apparent that between July 1979 and December 1980 the potentiation of muscimol by flurazepam increased significantly and that this greater effect has since been sustained.

In the same experiments, the potency of picrotoxin as an antagonist of muscimol was assessed. Picrotoxin 3×10^{-5} M shifted the muscimol dose-response line to the right and in the July 1979 series of experiments this antagonism was reduced in the presence of 10^{-6} M flurazepam (Fig. 2). A similar observation was made with lorazepam in June 1980 [8]. In subsequent series of experiments with flurazepam, however, this reduction in picrotoxin potency was not apparent, a change which coincided with the greater degree of potentiation of muscimol by flurazepam (Fig. 1).

The interpretation of these results has been based on an assumption that picrotoxin antagonized muscimol without reducing the potentiating effect of flurazepam. If, however, the conclusion from the earlier series of experiments is correct, that flurazepam reduces picrotoxin potency, then the converse might be expected, that picrotoxin should reduce the potency of flurazepam. In that case, the true reference line against which the picrotoxin antagonism of muscimol should be measured in the presence of flurazepam would be to the right of the muscimol line in flurazepam alone. This would mean that the potency of picrotoxin in flurazepam has

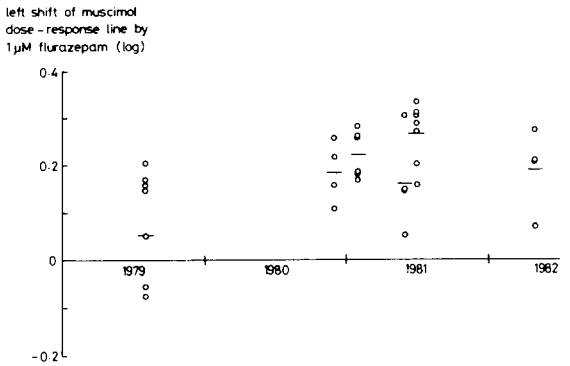

FIG. 1. Potentiation of muscimol by 10^{-6} M flurazepam in repeated series of experiments over 3 years. The potentiation was calculated from the left shift of the muscimol dose-response line and expressed in log units. Comparison of the data obtained in July 1979 with that obtained in December 1980 and January 1981 showed a significant increase (Student's t-test, $p<0.01$).

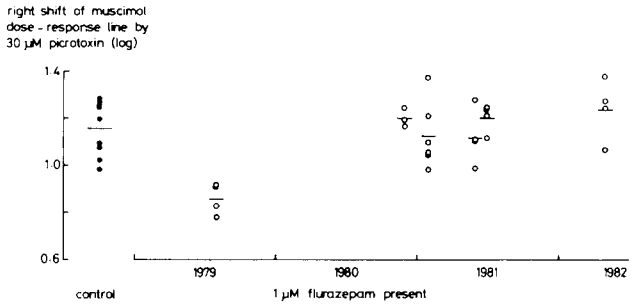

FIG. 2. Effect of 10^{-6} M flurazepam on the antagonism of muscimol by picrotoxin in repeated series of experiments over 3 years. Picrotoxin shifted the muscimol dose response line to the right and this is expressed in log units. In July 1979, flurazepam significantly ($p<0.01$), Student's t-test) reduced the potency of picrotoxin but from December 1980 onwards this effect was no longer apparent.

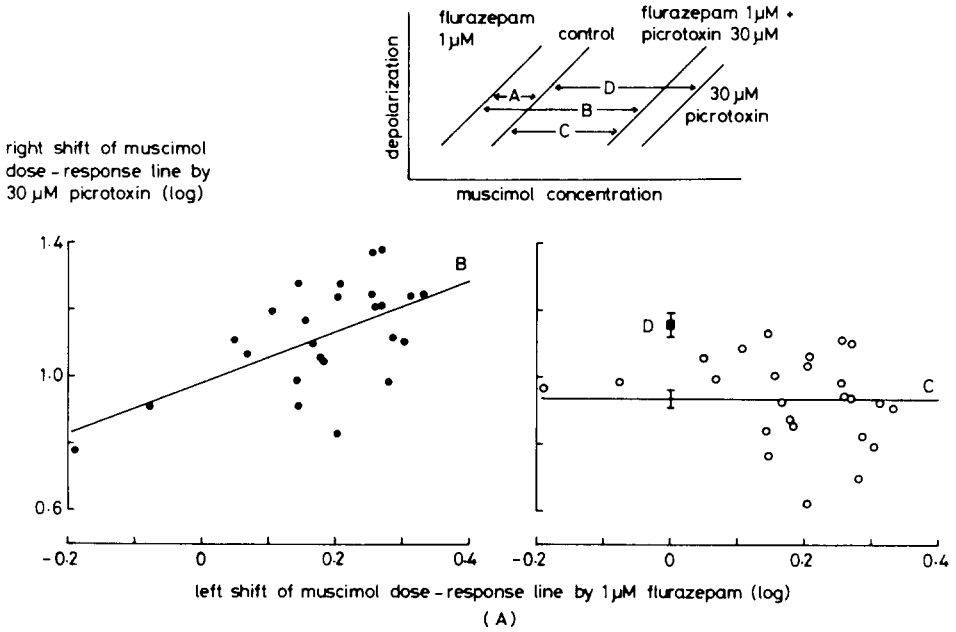

FIG. 3. Tests for correlation between reduction in picrotoxin potency by flurazepam and the potentiation of muscimol by flurazepam in the same experiment. The top part of the figure gives the key to the measurements plotted in the lower part of the figure. The data are taken from the same experiments as Figs. 1 and 2. When the muscimol dose-response line in 1 μM flurazepam was used as the reference against which the effect of 3×10^{-5} M picrotoxin in flurazepam was measured (B), the apparent potency of picrotoxin was significantly and positively correlated with the potentiation of muscimol by flurazepam (A) ($r=0.59$, $p<0.01$, Bravais-Pearson test). When the control muscimol line was used as the reference for the effect of picrotoxin in flurazepam (C), there was no correlation with the potentiation of muscimol by flurazepam (A) ($r=-0.21$, $p>0.05$) and the mean shift to the right of the muscimol dose-response line by picrotoxin was significantly less ($p<0.01$, Student's t-test) than that achieved by picrotoxin in the absence of flurazepam (D).

been overestimated and that the greater the potentiation of muscimol by flurazepam, the greater would be the overestimate of picrotoxin potency.

To examine this possibility, the antagonism of muscimol by picrotoxin 3×10^{-5} M in the presence of 10^{-6} M flurazepam was tested for correlation with the initial potentiation of muscimol by flurazepam in the same experiments (Fig. 3). The effect of picrotoxin was measured against each of two different reference lines: (1) the muscimol dose-response line in the presence of flurazepam, i.e., the same

reference line as used for the data in Fig. 2; (2) the original muscimol dose-response line in Krebs medium. The assumption implicit in the use of (1) was that the potentiation of muscimol by flurazepam was not affected by picrotoxin, whereas the use of (2) implied that the potentiation was completely abolished by the fairly high dose of picrotoxin. The antagonism of muscimol by picrotoxin based on (1) was positively correlated with the initial potentiation of muscimol by flurazepam; thus, the effect of picrotoxin was more likely to appear reduced by flurazepam in those experiments in which the potentiation of muscimol by flurazepam was small. When based on (2), the antagonism of muscimol by picrotoxin was not significantly correlated with the potentiation of muscimol by flurazepam and the mean antagonism was significantly less than that observed in the absence of flurazepam. A similar pattern could be seen within the individual series of experiments contributing to this overall analysis.

It would appear, therefore, that a reproducible reduction in picrotoxin potency by flurazepam can be inferred only if it is accepted that picrotoxin substantially reduces the potentiating effect of flurazepam. Such mutual antagonism of each others effects on the GABA system is an attractively simple explanation of the nature of the variability observed in these experiments but it does not indicate why the variability in response occurred. In contrast to this variability in benzodiazepine effect, the potency of picrotoxin alone as an antagonist of muscimol has shown no trend over several years [8]. It is possible that the effectiveness of flurazepam in an individual experiment could be influenced by the level of any endogenous activation of the benzodiazepine receptors. An attempt to model such a situation was made by repeating the previous experiments in the presence of a background level of 10^{-7} M flurazepam. The variability of the results was no different from that observed before, so it seems that the variations between experiments are unlikely to be due to variations in the level of endogenous activation of receptors. An alternative explanation could involve variations in the coupling of benzodiazepine receptors to the GABA receptor and ionophore complex but that remains to be investigated.

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