British Journal of Pharmacology (2006) 147, 237–238
www.nature.com/bjp

COMMENTARY

Modulating GABA modulators

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Benzodiazepines produce a broad spectrum of behavioral effects, which may be clinically desirable or undesirable, by positively or negatively modulating the effects of GABA at $GABA_A$ receptors. Over the past 20 years, much effort has been devoted towards identifying new compounds with limited undesirable effects. Most of this work has focused on developing drugs either with lower intrinsic activity than drugs such as diazepam, or with different binding profiles at subtypes of $GABA_A$ receptors. However, the benzodiazepine binding site is only one of multiple binding sites contained within the $GABA_A$ receptor complex, and other endogenous or exogenous compounds also may positively or negatively modulate the effects of GABA. Despite the availability of ligands for each of these distinct binding sites, very little research has examined the effects of GABA modulators given in combination. This may be due, in part, to the noncompelling results of those few studies which, depending on the particular drugs, have demonstrated site-selective antagonism or only additive effects, suggesting that each site modulates the effects of GABA independently. In this issue, McMahon and France challenge this view by showing that low-efficacy benzodiazepine ligands will effectively antagonize midazolam and, at the same doses, will enhance the effects of a neuroactive steroid. These studies raise interesting questions regarding the nature of the interaction between the benzodiazepine and neurosteroid binding sites on $GABA_A$ receptors.
British Journal of Pharmacology (2006) **147,** 237–238. doi:10.1038/sj.bjp.0706552;
published online 5 December 2005

Keywords: Benzodiazepine; neuroactive steroid; $GABA_A$; drug interactions

The $GABA_A$ receptor provides a complicated pharmacology. It is a pentameric structure that forms a ligand-gated Cl^- channel containing distinct binding sites for GABA, barbiturates, benzodiazepines, convulsants, and neuroactive steroids. Ligands that bind to these sites may directly open or block the Cl^- channel, though benzodiazepines, neuroactive steroids, and barbiturates act primarily by allosterically modulating the effects of GABA. There is also evidence that neuroactive steroids can increase the affinity of the benzodiazepine [^{3}H]flunitrazepam, for its binding site (Hawkinson *et al*., 1994). However, it is not known whether the converse is true, that is, that benzodiazepines enhance the binding of neurosteroids. In view of the intriguing pharmacology of $GABA_A$ receptors, and the common actions of neurosteroids and benzodiazepines, surprisingly few studies have pursued the effects of GABA modulators given in combination. In this issue, McMahon & France (2005b) convincingly demonstrate *in vivo* that benzodiazepine partial agonists are able to enhance the behavioral effects of a neuroactive steroid. With these results, McMahon and France advocate new avenues of research, proposing that low efficacy modulators at one site (benzodiazepine) may enhance the effects of positive $GABA_A$ modulators working at another site (neurosteroid).

McMahon & France (2001; 2005a) have previously addressed the topic of benzodiazepine-neurosteroid interactions by examining effects of neurosteroids and benzodiazepine ligands given in combination to rhesus monkeys. The results obtained in those earlier studies are consistent with the few other studies that have explored this issue. Thus, there is some agreement that positive modulators at the two sites have additive effects (Visser *et al*., 2003; McMahon & France, 2005a); antagonists selectively block drugs within the same class of compound (Cottrell *et al*., 1987; Mennerick *et al*., 2004); and negative modulators nonselectively decrease the effects of positive modulators (Brot *et al*., 1997; McMahon & France 2001). The distinction of the current studies by McMahon and France is that they used three benzodiazepine ligands that can be characterized as low efficacy partial agonists based on their positive modulation of GABA stimulated Cl^- currents. With these drugs, they demonstrate that drugs with little efficacy at benzodiazepine sites of $GABA_A$ receptors are able to dose-dependently antagonize the effects of a benzodiazepine, midazolam, in a primate species and, at the same doses and in the same subjects, enhance the midazolam-like effects of a neuroactive steroid, alfaxolone.

Among the different classes of drugs that can modulate GABA activity, the benzodiazepines have garnered the most attention. Benzodiazepine ligands may be either positive or negative allosteric modulators of GABA and are able to produce diverse behavioral effects that may be clinically desirable or undesirable, depending on the situation. Not all benzodiazepine agonists produce the same constellation of effects, however, and two views prevail in explaining observed differences among them. One view holds that drugs bind differentially to putative $GABA_A$ receptor subtypes, which in turn selectively mediate benzodiazepine effects (Klepner *et al*., 1979). The identification of 18 distinct proteins that combine to form the pentameric $GABA_A$ receptor has been regarded as strong evidence that multiple receptor subtypes may exist and studies with recombinant receptors in cell preparations or

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genetically modified mice has fueled hope that specific $GABA_A$ receptor subtypes might be associated with the mediation of particular effects of commonly used therapeutic drugs. Alternatively, it has been suggested that, as in other drug classes, benzodiazepines have different intrinsic activities and different behavioral effects may result from maximal or submaximal levels of receptor activation (Haefely *et al*., 1984). Of course, these two points of view are not mutually exclusive. As shown by Smith *et al*. (2001), different drugs can have both differing affinities and differing *in vitro* efficacies at atleast five recombinant $GABA_A$ receptor subtypes. Accordingly, as yet, there is no reason to discard or, for that matter, to favor either opinion. Nonetheless, McMahon and France embrace the view that efficacy is a more important determinant of drug action *in vivo*, and their data support this position.

The three benzodiazepine partial agonists used by McMahon and France have different effects in modified mouse cells, and the ability to positively modulate GABA may be determined by the subunit composition of recombinant $GABA_A$ receptors. This *in vitro* differentiation seems not to have had an impact *in vivo*, as all three drugs have qualitatively similar effects as benzodiazepine antagonists and as enhancers of the effects of alfaxolone. Thus, McMahon and France discuss their results almost solely in terms of the efficacy of the benzodiazepine ligands as positive GABA modulators. They suggest that even a slight enhancement of GABA binding (such as might be produced by flumazenil) is adequate for increasing the effects of steroid-induced increases in GABAergic effects, and they conclude that the positive modulatory actions of some otherwise-silent, low efficacy benzodiazepines may become evident when combined with positive $GABA_A$ modulators that act at a nonbenzodiazepine site. This is a provocative concept that promises to refresh the field of $GABA_A$ research. Of course, an alternative hypothesis might open the possibility that, in addition to allosteric modulation of GABAergic actions, benzodiazepine ligands also modulate the effects of neurosteroids more directly at their binding site on the $GABA_A$ complex.

Whether drugs that bind selectively to the benzodiazepine or neurosteroid site directly modulate actions of the other site, or whether their actions only meld at the level of GABAergic activity, remains to be seen. Of equal importance, the extent to which the obtained results can be generalized to other therapeutic consequences of GABAergic modulation, such as production of sedative, anxiolytic, or muscle relaxant effects, must still be determined. The paper by McMahon and France sets the occasion for further exploration of these questions and will hopefully encourage further study of the effects of GABA modulators given in combination.

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(Received October 5, 2005
Revised October 28, 2005
Accepted October 30, 2005
Published online 5 December 2005)