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Research Report

Intra-periaqueductal gray matter injections of midazolam fail to alter anxiety in plus-maze experienced mice

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ABSTRACT

It is well known that prior experience to the elevated plus-maze increases the avoidance of rodents to the open arms and impairs the anxiolytic-like effect of benzodiazepines evaluated during a subsequent exposure to the maze, a phenomenon known as "one-trial tolerance". Centrally injected benzodiazepine drugs attenuate anxiety in some limbic structures, such as hypothalamus, amygdala and the midbrain periaqueductal gray (PAG). This study investigated the effects of intra-PAG infusions of midazolam (MDZ) in maze-naïve and maze-experienced mice. The antiaversive effects of MDZ (3.0 nmol and 30 nmol in 0.1 μl) were evaluated by prior injection of flumazenil (16 nmol/0.1 μl), a benzodiazepine receptor antagonist, into the same midbrain site. Test videotapes were scored for conventional measures of anxiety and locomotor activity, as well as a range of ethological measures related to risk assessment. In maze-naïve mice, intra-PAG infusions of MDZ increased % open arm entries (3.0 nmol) and % open arm time (3.0 and 30 nmol). These effects were observed in the absence of significant changes in locomotor activity, indicating a selective anxiolytic-like effect of MDZ. The antiaversive effects of MDZ were completely blocked by prior injection of flumazenil which in turn did not alter any other behavioral measure. In maze-experienced mice, intra-PAG infusion of MDZ did not modify any behavioral measure. Taken together, present results corroborate previous studies demonstrating that GABA/benzodiazepine receptor complex located within the PAG plays a role on anxiety modulation in maze-naïve mice as well as indicate its involvement in the OTT phenomenon.

1. Introduction

Administration of benzodiazepines usually produces anxiolytic effects in animal models of anxiety such as social interaction test (for review see File and Seth, 2003), light–dark exploration (for review see Hascoët et al., 2001), open-field (for review see Prut and Belzung, 2003), shock-probe burying (De Boer and Koolhaas, 2003), Geller conflict (Babbini et al., 1982; Howard and Pollard, 2004; Kennett et al., 1995, 1998; Pietraszek et al., 2005; Smith and Barret, 1997), Vogel conflict (Agmo et al., 1995; Millan, 2003) and elevated plus-maze (Cole and Rodgers, 1993; Griebel et al., 1996; Lister, 1987; Nunes-de-Souza and Rodgers, 2000; Rosa et al., 2000; Treit, 1991).

Since its introduction (Handley and Mithani, 1984), the elevated plus-maze (EPM) became one of the most used animal models to detect the anxiolytic activity of drugs (Hogg, 1996). The test is based on the rodent natural aversion to open spaces

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(Fernandes and File, 1996, Treit et al., 1993) and was validated in rats (Pellow et al., 1985) and mice (Lister, 1987; Stephens et al., 1986). The main index of anxiety in the elevated plus-maze is related to space and time measures of open arms avoidance, whereas the locomotor activity is evaluated by the frequency of closed arms entries (File, 1992; Lister, 1987). An increase in the open arm activity (e.g., increase in percentage of open arm entries or percentage of open arm time) indicates reduction of anxiety, whereas changes in the number of entries in closed arms indicate non-specific effects in the locomotor activity (Menard and Treit, 1999). In the EPM, animals also exhibit a set of behavioral responses (e.g. stretched attend postures, head dipping) to gather information regarding the potential threat of the open arms. This behavioral strategy has been characterized as risk assessment behavior (Blanchard and Blanchard, 1989; Cruz et al., 1994; Rodgers et al., 1997). Importantly, the efficacy of the EPM in discriminating anxioselective compounds has been increased with the adoption of a more ethological analysis (Cruz et al., 1994; Rodgers and Johnson, 1995; Carobrez and Bertoglio, 2005).

An important factor of the EPM is related to the effect on the previous experience to the test. A single experience in the maze usually decreases open arm exploration during a second trial. Interestingly, anxiety-related behaviors recorded during trial 2 are also insensitive to anxiolytic drugs (File and Zangrossi, 1993; Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993). Although maze experience usually increases open arm avoidance during a second trial (Bertoglio and Carobrez, 2000; Dawson et al., 1994; Fernandes and File, 1996; Gonzalez and File, 1997; Holmes and Rodgers, 1998; Holmes and Rodgers, 1999; Rodgers and Shepherd, 1993; Rodgers et al., 1996; Treit et al., 1993), previous findings have also failed to detect changes in anxiety indices in subsequent trials (File et al., 1990; Lister, 1987; Pellow et al., 1985).

The failure of well-known anxiolytic compounds (e.g. benzodiazepines) to attenuate anxiety indices in maze-experienced animals has been interpreted as one-trial tolerance (OTT) (Bertoglio and Carobrez, 2002a; File 1990; File et al., 1990; Holmes and Rodgers, 1999; Lister, 1987; Rodgers and Shepherd, 1993). Evidence that OTT also happens with other anxiolytic compounds has been demonstrated (for a review see Carobrez and Bertoglio, 2005). In addition, OTT seems to be independent of pharmacological treatment

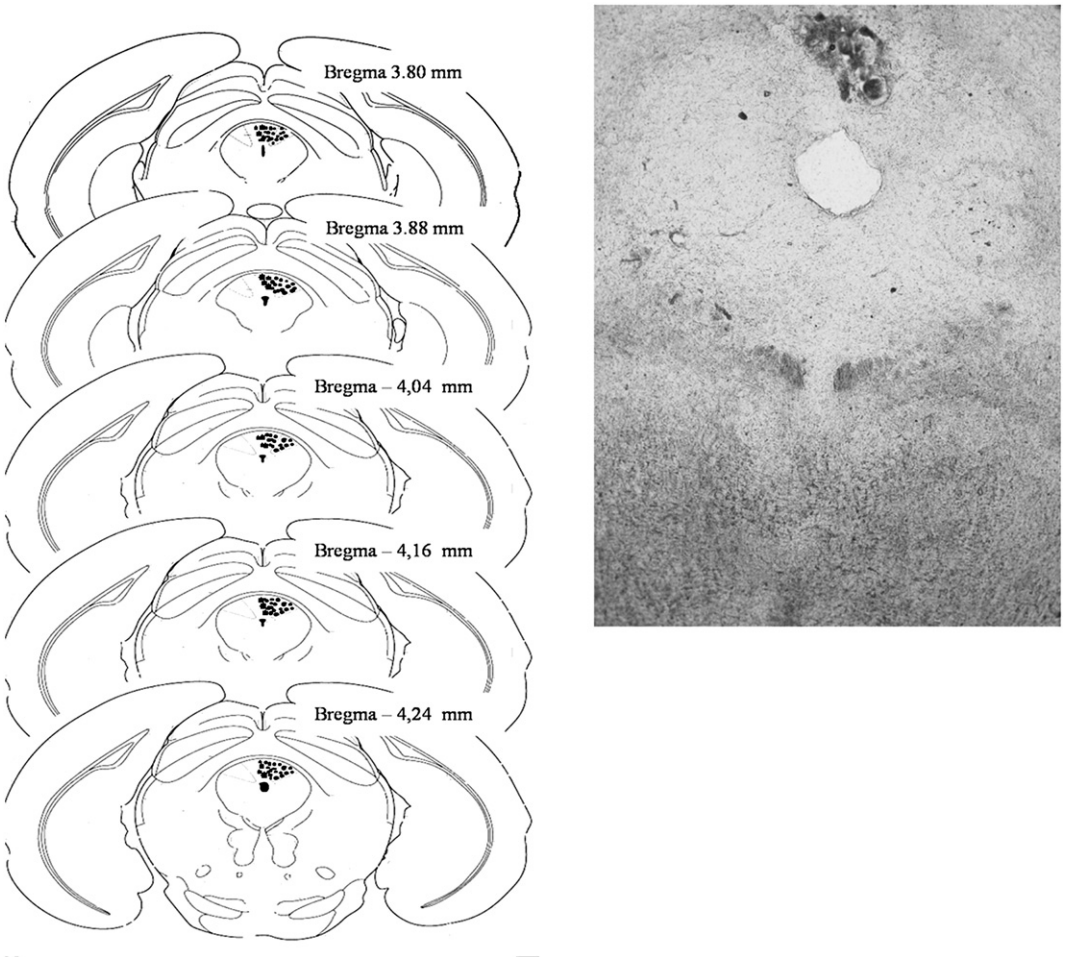

Fig. 1 – Schematic representation of microinfusion sites within the midbrain periaqueductal gray (PAG) of the mice (A). The number of the points in the figure is less than the total number of mice because of the overlaps. (B) Photomicrograph of midbrain coronal section from a representative subject showing an injection site into the PAG. Section corresponds to –4.24 mm from bregma in the atlas of Franklin and Paxinos (1997).