DOSE RELATED ANXIOLYTIC EFFECTS OF DIAZEPAM: RELATION WITH SERUM ELECTROLYTES, PLASMA OSMOLALITY AND SYSTOLIC BLOOD PRESSURE (SBP) IN RATS

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ABSTRACT
Diazepam is an anxiolytic and anticonvulsant drug that also induces hypnosis. Changes in serum electrolyte balance, plasma osmolality and systolic blood pressure (SBP) are often associated with stress-induced anxiety. Administration of diazepam has been shown to decrease stress-induced enhancement of hypothalamic pituitary adrenal cortical (HPA) axis. The present is designed to monitor the anxiolytic effects of different doses of diazepam (1mg/kg, 2.5mg/kg and 5mg/kg) and its association with changes of serum electrolyte balance, plasma osmolality and SBP in rats. Administration of diazepam at doses of 1mg/kg, 2.5mg/kg and 5mg/kg elicited anxiolytic effects monitored in light-dark transition test and increased serum concentration of electrolytes and plasma osmolality. Serum levels of magnesium as well as SBP decreased. The results are discussed in context of anxiolytic effects of diazepam to be mediated via a modulation of stress-induced increase in the activity of HPA-axis and electrolytes balance.

INTRODUCTION
Diazepam and other benzodiazepines are used to reduce stress-induced anxiety (Liberzon et al., 2003; Griebel et al., 1998; Attack, 2003). In animals, benzodiazepines appears to act on parts of the limbic system to elicit calming effects (Pomara et al 2004; Myrray, 1990; Bhattacharayya, 1997; Liebsch et al., 1998a, Pivac and Pericic, 1993). At molecular level benzodiazepines elicit hyper polarization by increasing the inhibitory effect of GABA (Dhawan et al., 2003; Glordano et al., 2003). These drugs also decrease the sympathetic activity (Vitela et al., 2005; Marcus et al., 2002). Changes of electrolytes are involved in stress-induced hypertension (Mehboob et al 1994).Therefore it may be expected that diazepam and other benzodiazepines elicit anxiolytic effects and decrease SBP by changing electrolyte balance. The present study therefore concerns with dose related effects of diazepam on serum electrolytes plasma osmolality and SBP in rats. Anxiolytic effects of drug are also monitored in light-dark activity box.

MATERIAL AND METHODS

Animals
Male albino Wistar rats (n=24) weighing 175-250g purchased locally were caged individually in a quiet and temperature controlled room (25±4°C) for 3 days. Rats had free access to water and standard rodent diet.

Drug
Diazepam (Merck brand) purchased locally in the form of ampoules (10mg/2ml) was injected i.p. at doses of 1, 2.5 and 5mg/kg. Control animals were injected with deionized water (1ml/kg).

Experimental protocol
Male albino Wistar rats (n=24) were injected diazepam i.p at doses of 1mg/kg, 2.5mg/kg and 5mg/kg. Control animals were injected with deionized water (1ml/kg). The animals injected with water or buspirone were kept back in their home cages. Light- dark box activity was monitored for 5 minutes starting 45 minutes post injections. SBP of rats were also noted 45minutes post injection. Animals
were decapitated 1hr post injection to collect blood in non-heparinized and heparinized tubes for the analysis of serum electrolytes and plasma osmolality respectively.

**Light-dark box activity**

Light-dark activity box was conducted in locally made two-compartments box. The compartments of equal size (26x26x26), with an access (12x12cm) between the compartments, differed in their sensory properties. Walls of one compartment were light (transparent) and other dark (Black). A rat introduced into the box via placing it in the light box. Time pass in the light compartment and number of entries in light compartment were monitored for a cut off time of 5 minutes starting 45 minutes post injection.

**Systolic blood pressure (SBP) measurements**

SBP was measured by using NIBP (non-invasive blood pressure) controller (ML0126) attached to the recorder. Rats were restrained in a clear, plastic tube at 39 C, and the cuff was placed on the tail and inflated to 200 mm Hg. The reappearance of a pulse during deflation of the cuff was used to determine SBP. To minimize stress, no animal was restrained for more than 10 min at a time, and a minimum of three clear SBP recordings were taken per animal.

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**Fig. 1:** Dose related effects of diazepam on number of event (A), and time spent (B) of rats. Values are X±S.D. Significant difference by Newman-Keuls test, *p<0.01 with respect to water treated rats, following one-way ANOVA.
Analytical methods

Serum sodium, calcium and potassium were estimated by a flame photometer (Corning 410c). Concentration of magnesium in serum was estimated by the method describe earlier by Hallry and Sky peck (1964). Plasma osmolarity of drugs treated and untreated animals were determined by direct Cryoscopic osmometer (Osmomat-030).

Results are represented as means±S.D. Data were analyzed by one-way ANOVA. Significant results were further compared by Newman-Keuls test.

RESULTS

Dose related effects of diazepam on number of events and time spent in light compartment of light-dark activity box

Figs. 1A and B show the effects of diazepam at doses of 1, 2.5 and 5 mg/kg) on number of events and time spent. Results show significant effects of diazepam on number of events (F=663.6 df3,20 p<0.01) and time spent (F=859 df3,20 p<0.01) in light compartment of light-dark activity box. Post-hoc analysis revealed that doses of diazepam between 1-
Dose Related Anxiolytic Effects of Diazepam

5mg/kg produced dose dependent increase in number of events and time spent in light box.

Dose related effects of diazepam on serum sodium, potassium and SBP of rats

Figs. 2A, B and C. shows the effects of diazepam at doses of 1, 2.5 and 5mg/kg on serum sodium, potassium and SBP of rats. One way ANOVA showed significant effects of diazepam on concentration of sodium (F=8.12 df3, 20 p>0.01), potassium (F=8.5 df3,20 p<0.01), and SBP (F=26 df3,20 p<0.01) Post hoc analysis showed that doses of diazepam between 1-5mg/kg produced significant (p<0.01) increase in sodium, potassium and decrease in SBP of rats. The increases of sodium and potassium and decrease of SBP were dose dependent.

Dose related effects of diazepam on serum calcium, magnesium and plasma osmolality of rats

Fig. 3 (A, B and C) shows the effects of different doses of diazepam on serum calcium, magnesium levels and plasma osmolality. One way ANOVA revealed significant effects of
diazepam on calcium (F=42.5 df3, 20 p<0.01), magnesium (F=293 df3,20 p<0.01) and plasma osmolality (F=9.2 df3, 20 p<0.01). Newman-Keuls test showed that administration of diazepam increased serum calcium, plasma osmolality and decreased magnesium concentration in a dose dependent manner.

**DISCUSSION**

Results of present investigation demonstrated that administration of diazepam (1mg/kg, 2.5mg/kg and 5mg/kg) elicited dose dependent antianxiety effects in light-dark activity box. Previous studies have also reported that diazepam at doses between 1-10mg/kg concentration (Choulff et al., 1997; Greibel et al., 1998) increased the number of entries and time duration in light box. Diazepam decreased the latency to enter the light compartment. Hascoet & Bourin (1998) reported that mice given diazepam were able to cross over the brightly lit area more quickly and more often and spend more time there than do control mice.

The increases in sodium, potassium and calcium and decreases in magnesium and SBP in rats treated with diazepam (Figs. 2 and 3) as observed in the present study are explainable in term of decrease in aldosterone and catecholamines in diazepam treated animals (Glodano et al., 2003). Indeed it has been shown that diazepam administration could decrease the activity of hypothalamus pituitary adrenal axis (HPA) (Bateson, 2002; Kovacs et al., 2002; Mikkalsen et al 2005) and secretion of aldosterone and catecholamines (Glodano et al., 2003; Bhattacharyya and Sur, 1999; Hagret and Vogel, 1995; Breir et al., 1992). Smaller secretion of adrenal hormone may modify the membrane permeability for electrolytes to decrease intracellular Mg$^{2+}$/Ca$^{2+}$ shift and thus may decrease the levels of calcium and sodium (Ising et al., 1986). It could lead to the vasodilatation and reduction in systolic blood pressure. The observed increase in calcium and decrease in magnesium (Figs. 2 and 3) may also be due to an altered shift of these electrolytes at cellular levels. Administration of diazepam could decrease the membrane permeability of catecholamines sensitive cells, which in turn reduces calcium influx into cells to liberate intracellular magnesium (Ennaceur et al., 2006; Breir et al 1992; Zemmishiany et al 1990). It is suggested that diazepam induced decrease in the activity of HPA-axis modulate serum electrolytes to reduce systolic blood pressure.

**REFERENCES**


