CARDIOGRAM PATTERN AFTER SEDATIVE DOSE OF DIAZEPAM IN RABBITS

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ABSTRACT

In so-called anesthetic doses, all benzodiazepines decrease blood pressure and increase heart rate secondary to a decrease in peripheral resistance. But exceptionally, with diazepam they are reported to be secondary to a decrease in left ventricular work and cardiac output. This study was conducted to determine the effect of diazepam in subanesthetic dose on the different phases of cardiac work; the rapid, reduced ejection phases of ventricular systole and on the arterial pressure decay after aortic valve closure. These phases of cardiogram were recognized and analyzed using a computer system connected to two biopreamplifiers for arterial pressure and ECG monitoring simultaneously. Single arterial pressure wave could be magnified for doing different estimations for the different

phases of the arterial pressure wave. Two groups of rabbits, each of 6 animals were used to test the effect of diazepam (0.7 mg /rabbit i.v) and the blocking or reversing effects induced by flumazenil (0.02 mg /rabbit i.v.). Each animal served as a control for itself.

Diazepam and not flumazenil decreased the ejection period as compared with that of control (0.56 ± 0.09 Vs 1.00 ± 0.09 msec). This effect was not accompanied by any change in heart rate and cardiac cycle period. Flumazenil blocked this diazepam effect but insignificantly reversed it. Increased ejection velocity by diazepam was indicated by an increase in the ascending limb slope of the arterial pressure wave (0.6060 ± 0.0367 Vs 0.4845 ± 0.0279) together with unchanged pulse pressure. Flumazenil

had significantly blocked and significantly reversed this effect of diazepam. Meanwhile, diazepam had increased the slope of the second part of the descending limb of the wave corresponding to the isometric ventricular diastole. This could be consequent to the earlier closure of the aortic valve. It indicates prolongation of the high-pressure period of post-aortic valve closure, which could be beneficial for coronary artery filling. Flumazenil had significantly blocked and reversed this effect of diazepam. The potential relevance of these findings for different cardiac patients could be in favor of cardiac performance and coronary artery filling by diazepam in subanesthetic dose.

Key words:

Diazepam - Flumazenil - Cardiogram.

INTRODUCTION

The benzodiazepine derivative, diazepam is frequently used to induce anesthesia or to produce sedation. In anesthetic doses, all benzodiazepines decrease blood pressure and increase the heart rate. With flunitrazepam and midazolam, the effects are secondary to a decrease in peripheral resistance (Seitz et al., 1977), but

with diazepam and lorazepam these effects are secondary to a decrease in left ventricular work and cardiac output (Rao et al., 1973 and Al-Khudhairi et al., 1982).

This cardiac effect of diazepam has to be declared with its sedative dose, since diazepam is very popular in cardiological practice for producing mild sedation and relief of emotional tension. It has been used for premedication before cardiac catheterization (Tornetta, 1965) and for DC cardioversion (Nutter & Massumi, 1965 and Somers et al., 1971).

In this study, the cardiac efficiency under diazepam sedative dose was evaluated by the cardiogram recognized through peripheral artery pressure wave (Remington and O'Brien, 1970). The degree of involvement of benzodiazepine receptors has been tested using the specific antagonist flumazenil.

MATERIALS AND METHODS

Twelve males conscious rabbits of 1.5 ± 0.12 Kgm body weight each were used divided into two equal group. Each animal served as a control for itself. The cardiogram was recorded through an arterial cannula inserted

via the central ear artery. Its pattern through a peripheral artery is similar to that recordable directly through the aorta (Remington and O'Brien, 1970). Additionally, the timing of aortic valve closure could be fixed on this peripheral arterial pressure wave (Fig. 1).

The estimated different cardiac wave parameters reflecting the cardiac work were, the total ejection period (rapid and reduced ejection phases), the phase of arterial pressure decay after aortic valve closure and the cardiac ejection velocity (dp/dt) indicating the slope of the ascending limb. The timing of aortic valve closure was indicated by a notch on the descending limb (Bern and Levy, 1998a). The termination of the total ejection period was indicated by the notch at the upper half of the descending limb.

These cardiographic parameters were recorded and analyzed using a computer system connected with two bio-preamplifiers for simultaneous arterial pressure and ECG monitoring (Intracel Physiology Teaching System: Intracel). Six bands of records of two minutes duration were saved to be analysed later on for each test interval. These intervals correspond to; the premedication period, after the fi-

rst drug and after the second drug administration. A period of ten minutes was permitted to gain blood pressure stability before the intended two minutes recording. This pause of ten minutes also allows maximum efficacy of both diazepam and flumazenil (Brogden and Goa, 1991).

Single arterial pressure wave was magnified through a fixed window for estimating the data for all animals. Average estimate of 6 measurements was calculated inside each interval for each parameter for each rabbit.

The first group of animals received first diazepam in a sedating dose of 0.7mg i.v./rabbit of 1.5 Kgm body weight (Hobbs et al., 1996). Fifteen minutes later, flumazenil in a dose of 0.02 mg/rabbit was i.v. injected to test the reversibility of diazepam effects.

The second group received flumazenil fifteen minutes before diazepam to test also the blockability of its probable effects (Hobbs et al., 1996).

Diazepam sedating dose was determined after transformation to adapt the species of rabbit. Also flumazenil dose was similarly determined after estimating its proportionality to dia-

zepam sedating dose.

Analysis of our data was performed through Mann-Whitney test.

RESULTS

The diazepam and flumazenil doses had no effect on the heart rate. This is indicated by the non-significant difference between the R-R intervals estimated through the ECG records in the two groups of rabbits (Table 1). They did not significantly change the arterial pressure and pulse pressure difference.

Diazepam increased the slope (dp/dt) of the ascending limb of the arterial pressure wave if compared with the control value (0.6060 ± 0.0367 Vs 0.4845 ± 0.0279 mmHg/msec. (Table 1, Fig. 1). This effect was not significantly reversed by flumazenil. While prior infusion of flumazenil had decreased significantly this slope compared with the control value

(0.3418 ± 0.0196 Vs 0.4845 ± 0.0279 mmHg/msec.). The latter effect appears to be significantly reversed by subsequent infusion of diazepam (table 1). But diazepam value was not mounting to that of diazepam of the first group.

Meanwhile, diazepam had decreased significantly the ejection period; an effect which was not reversed by flumazenil, nor significantly blocked by prior flumazenil infusion in the second group. The slope of the descending limb after aortic valve closure, denoting the arterial pressure decay, was significantly increased by diazepam. It was reversed to control level by the subsequent infusion of flumazenil (Table 1 & Figs. 1,2).

Contrary to diazepam, flumazenil prior infusion in the second group of rabbits had decreased this slope. Diazepam in the latter group had no significant effect after flumazenil.

Table (1) Effect of diazepam and flumazenil on the cardiogram recognized through the arterial pressure wave ($m \pm sem$).

	Pretreatment level (n=12)	First group (n=6)		Second group (n=6)	
		Diazepam 1st.	Flumazenil 2nd	Flumazenil 1st.	Diazepam 2nd
MBP (mmHg)	100 $\pm$ 10	90 $\pm$ 7	96 $\pm$ 9	104 $\pm$ 6	98 $\pm$ 12
R-R interval (sec).	0.22 $\pm$ 0.03	0.20 $\pm$ 0.03	0.22 $\pm$ 0.03	0.19 $\pm$ 0.02	0.19 $\pm$ 0.03
Ejection period (msec).	1.00 $\pm$ 0.09	0.56 $\pm$ 0.09*	0.67 $\pm$ 0.08*	0.98 $\pm$ 0.13†	0.77 $\pm$ 0.20
dp/dt of the ascending limb of pulse wave (mmHg/msec.)	0.4845 $\pm$ 0.0279	0.6060 $\pm$ 0.0367*	0.5620 $\pm$ 0.0376	0.3418 $\pm$ 0.0196*†	0.4407 $\pm$ 0.0333#†
dp/dt of the descending limb after aortic valve closure (mmHg/msec).	0.1020 $\pm$ 0.0062	0.2020 $\pm$ 0.0097*	0.0905 $\pm$ 0.0065†	0.0784 $\pm$ 0.0063*†	0.0619 $\pm$ 0.0184*†

Mann-Whitney test with significance when $p < 0.05$

- * Significance of difference between pretreatment and treated groups
- † Significance of difference between 1st diazepam group and the others.
- # * Significance of difference between 1st Flumazenil group and the others

Fig (1) : Showing the effect of diazepam on cardiogram of central ear artery of rabbits (Dotted line: Diazepam effect).

Fig (2) : Effect of diazepam and flumazenil on the slope (dp/dt) of the descending limb of the arterial pressure wave (* Vs. pretreatment and † Vs. diazepam 1st).

DISCUSSION

Our experiments show that diazepam in the selected sedative dose did not produce any significant change neither in heart rate nor in blood pressure. This is in accordance with the results obtained by Ikram and Rubin, (1971) in human using the equivalent dose used in the present work.

The results point out to a significant increase in the slope (dp/dt) of the ascending limb of the pulse wave. They indicate a significant increase in

the ejection velocity of the left ventricle (Berne and Levy, 1998a); Ikram and Rubin (1971) pointed to a non-significant change of the right ventricular dp/dt in their cardiac patients. This effect of diazepam in our results was associated with a significant shortening in the ejection period with consequent earlier closure of the aortic valve. This closure was identified by a corresponding notch on the descending limb (Berne and Levy, 1998a). The previous two findings of increased ejection velocity and the earlier

closure of the aortic valve indicate more efficient left ventricular function under diazepam sedating dose in normal rabbits. These findings are in contradiction to those reported by Rao et al., 1973 and Al-Khudhairi et al., 1982 with diazepam anesthetic dose; of a decrease in left ventricular work and cardiac output.

Closure of the aortic valve was followed by a significant delay in the decay of the arterial pulse pressure, which is indicated by the greater slope of the pulse wave descending limb measured after aortic valve closure. This could be consequent to both increased ejection velocity of the left ventricle together with the earlier closure of the aortic valve.

This action of diazepam can assure better coronary artery filling during the diastolic period. Coronary blood flow proportionates to the aortic pressure level along its decay after aortic valve closure (Berne and Levy, 1998b). The coordination between aortic valve closure and aortic pressure decline rate has to be explored. Recently, through an-isolated perfused heart, it is reported that sinus

node discharge is mechanically coupled to coronary perfusion on a beat-to-beat basis (Slovut et al., 1999). Heart rate accelerated as coronary artery perfusion pressure was increased.

There is a certain amount of evidence from animal studies that diazepam increases coronary blood flow in the isolated canine heart preparation (Abel et al., 1970a,b and Taylor et al., 1970). In man Ikram et al. (1973) showed that diazepam in a sedative dose, induced 22.5% increase in myocardial blood flow in patients with normal coronary arteries and 73% increase in patients with diseased coronary arteries.

Abel et al. (1970a) concluded that their finding could not be due to extracardiac humoral materials or to reflexes mediated via the effect of diazepam on the central nervous system; since these changes of increased coronary blood flow also occurred in the isolated heart. In the other study, Abel et al. (1970b) found that diazepam induced coronary vasodilatation was completely opposed by ganglion blockade with trimetaphan and concluded that

diazepam caused coronary vasodilatation by specifically stimulating vasodilating mechanism at the post-ganglionic neurone. However, the mediation of either cardiac or central benzodiazepine receptors cannot be denied. In our experiments, the selective benzodiazepine receptor blocker flumazenil reversed and blocked the outcome of diazepam on the cardiogram. Simultaneous to the discovery of binding sites for benzodiazepines in the central nervous system was the observation that (3H) diazepam also bound to sites in peripheral tissues including heart and kidney. Central and peripheral GABA and benzodiazepine receptors are extensively studied only for the kidney (Erd et al., 1991; Monasterolo et al., 1995; Drugan, 1996 and Martin & Haywood, 1998). Renal benzodiazepine receptors are under stress-induced regulation coupled with angiotensin II (Drugan, 1996). Their significance in the pathophysiology of hypertension has been suggested.

The suggested peripheral contribution of cardiac benzodiazepine receptors for the observed increased ejection velocity, the earlier aortic

valve closure and the delayed decay in aortic pressure cannot exclude their probable contribution for the peripheral resistance on the obtained results with diazepam and flumazenil.

Conclusion:

In conclusion, our results with diazepam sedating dose indicated an increased ejection velocity, shortening in the ejection period and slower decay in the arterial pressure after aortic valve closure. These effects could allow for better coronary filling for a probable consequent hypotonic ventricles during the diastolic period.

These observations need further confirmatory experiments exploring their potential relevance in cardiac patients exposed to stressful interventions.

REFERENCES

- Abel, R.M.; Reis, R.L. and Staroscik, R.N. (1970a) : Coronary vasodilatation following diazepam (Valium) Br. J. of Pharmacol, 38: 620.
- Abel, R.M.; Reis, R.L. and Staroscik, R.N. (1970b) : The phar-

macological basis of coronary and systemic vasodilator actions of diazepam (Valium). *Br. J. of Pharmacol*, 39: 261.

Al-Khudhairi, D.; Whitwam, J.G.;

Chakrabarti, M.K;

Askitopoulou, H.; Grundy,

E.M. and Powrie, S. (1982)

: Haemodynamic effects of midazolam and thiopentone during induction of anaesthesia for coronary artery surgery. *Br. J. Anaesth.*, 54: 831.

Berne, R.M. and Levy, M.N. (1998a)

: The cardiac pump-sec. IV. The cardiovascular system. Ch. 23. In *Physiology*, ed. Berne, R.M. and Levy, M.N. 4th edition, Mosby, New York, PP; 360.

Berne, R.M. and Levy, M.N. (1998b)

: Special criculation-sec. IV. The cardiovascular system. Ch. 30. In *Physiology*, ed. Berne, R.M. and Levy, M.N. 4th edition, Mospy, New York, PP; 478.

Brogden, RN. and Goa, KL. (1991) :

Flumazenil a reappraisal of its pharmacological properties and therapeutic efficacy as a benzodiazepine antagonist. *Drugs*, 42: 1061.

Drugan, RC. (1996) : Peripheral benzodiazepine receptors: molecular pharmacology to possible physiological significance in stress-induced hypertension. *Clin. Neuropharmacol*. 19: 6, 475.

Erd, S.L.; Dob, E.; Pdrucz, A. and

Wolff, J.R. (1991) : Releas-

able GABA in tubular epithelium of the rat kidney *Experientia*, 47: 3, 227.

Hobbs, W.R.; Rall, T.W., Verdoorn

T.A. (1996) : Hypnotics and Sedatives, In Goodman and Gilman's, *The Pharmacological Basis of Therapeutics* 9th. edition. McGraw-Hill ed. PP. 361 .

Ikram, H. and Rubin, A.P. (1971) :

Anaesthesia for cardioversion. *Br. Med. J.* 4: 626.

Ikram, H.; Rubin, A.P. and Jewkes,

- R.F. (1973)** : Effect of diazepam on myocardial blood flow of patients with and without coronary artery disease. *Br. Heart J.* 35; 626.
- Martin, D.S. and Haywood, J.R (1998)** : Reduced GABA inhibition of sympathetic function in renal-wrapped hypertensive rats. *Am. J. Physiol.* 275: 5 Pt 2, R: 1523.
- Monasterolo, L.A.; Ochoa, J.E.; Trumper, L. and Elyas, M.M. (1995)** : Vascular and tubular actions of diazepam in isolated and perfused rat kidney. *Eur. J. Pharmacol.*, 276: 3, 201.
- Nutter, D.O. and Massumi, R.A. (1965)** : Diazepam in cardioversion. *New England J. of Med.* 273: 650.
- Rao, S.; Sherbaniuk, R.W.; Prasad, K; Lee, S.J.K. and Sproule, B. J. (1973)** : Cardiopulmonary effects of diazepam. *Clin. Pharmacol. Ther.*, 14: 182.
- Remington, J.W. and O'Brien, L.J. (1970)** : Construction of aortic flow pulse from pressure pulse. *Am. J. Physiol.* 218; 437.
- Seitz, W.; Hempehnan, G.; and Piepenbrock, S. (1977)** : Zur Kardiovaskularen Wirkung Von. Flunitrazepam (Rohypnol Ro-5-4200) Anaesthetist, 26: 249. quoted from "Stewart C. Harvey : Hypnotics and Sedatives. In Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 7th ed. Macmillan ed. 1985, PP; 339.
- Slovut, D.P.; Wenstrom, J.C.; Moeckel, R.B.; Salerno, C.T.; Park, S.J. and Osborn, J.W. (1999)** : Beat-to-beat modulation of heart rate is coupled to coronary perfusion pressure in isolated heart. *J. Appl. Physiol.* 88(2): 694.
- Somers, K.; Gunstone, R.F.; Patel, A.K and D'Arbela, P.G. (1971)** : Intravenous diazepam for direct current cardio-

version. Br. Med. J.4: 13.

(Abstract). Pharmacologist,
12; 233.

Taylor, P.L.; Daniell, H.B. and Bagwell, E.E. (1970) : Studies on the myocardial hemodynamic and metabolic effects of diazepam in the dog

Tornetta, F.J. (1965) : Diazepam as pre-anaesthetic medication. Anesthesia and Analgesia. 44; 449.

ديناميكية القلب بعد جرعة مهدئة

من الديازيبام فى الأرناب

تؤدى أدوية البنزوديازيبين إلى إنخفاض ضغط الدم عند إستخدامها فى جرعات تخديرية ويعزى ذلك إلى نقص المقاومة لسريان الدم فى الأوعية الدموية. ولكن مع دواء الديازيبام فإنه يعزى إلى إنخفاض فى فاعلية البطين الأيسر ونقص فى معدل ضخ القلب للدم. ونظراً لكثرة إستخدام دواء الديازيبام فى جرعات أقل من الجرعات التخديرية (جرعات مهدئة) رؤى أن نقوم بدراسة تأثير هذه الجرعات على ديناميكية ضخ الدم بعضلة القلب. فقمنا بمتابعة مراحل الإنقباض والإسترخاء من خلال برنامج كمبيوتر وباستخدام وحدات خاصة لاستشعار نبضات ضغط الدم الشريانى وموجات رسم القلب متزامنين فى حين واحد. وهذا البرنامج يسمح بالتسجيل لفترات محددة واسترجاعها لتحليلها بعد إنتهاء التجربة. ويسمح أيضاً بتكبير النبضات على حدة وتحليل كل مرحلة من نبضة ضغط الدم. فى هذه التجربة قسمت الأرناب إلى مجموعتين كل منها تتكون من ستة أرناب وأعتبرت القراءات المسجلة لكل أرناب قبل حقن الدواء كأساس للمقارنات مع النتائج بعد حقن الدواء فى نفس الأرناب. وصممت التجربة بحيث يمكن إختيار تأثير دواء الديازيبام محقوناً بالوريد فى جرعة ٧ مجم/أرناب على ديناميكية نبضة ضخ الدم بالقلب وإختيار قدرة دواء الفلومازينيل (مضاد البيازيبام على مستقبلات) محقوناً بالوريد فى جرعة ٠.٢ مجم/أرناب على إسترجاع حالة الضخ رغم سابق حقن الديازيبام وذلك فى المجموعة الأولى من الأرناب. وفى المجموعة الثانية أختبرت قدرة الفلومازينيل على منع فاعلية الديازيبام على ضخ الدم بحقنه قبل الديازيبام. وأظهرت نتائج هذا البحث قدرة الديازيبام على إنقاص فسترة ضخ الدم بالبطين الأيسر وعدم قدرة الفلومازينيل على منع هذا التأثير. وفى نفس الوقت أدى الديازيبام إلى زيادة فى معدل الضخ متمثلاً فى زيادة درجة إنحدار الساق الصاعدة للنبضة 60.6 ± 36.7 ومقابل 45.8 ± 27.9 ر. للأساس على ثانية/مليمتر زئبق) وأدى الفلومازينيل إلى إسترجاع قيمة الأساس إذا حقن بعد الديازيبام وكذلك إلى منع تأثير الديازيبام إذا حُقن قبله. ومن ناحية أخرى أدى الديازيبام إلى زيادة فى معدل إنحدار الساق الهابطة للنبضة بعد غلق الصمام الأورطى، وقد يُعزى ذلك إلى الغلق المبكر للصمام. أدى هذا إلى طول مدة تواجد الدم الشريانى بضغط أعلى من مستوى الضغط الانبساطى الذى يضمن سريان الدم إلى الشريان التاجى بصورة أفضل. وأيضاً أدى الفلومازينيل إلى منع هذا التأثير وإسترجاع معدل الأساس. وهذه النتائج تدل على قيمة مستقبلات البنزوديازيبين لديناميكية عضلة القلب وفاعلية الدورة الدموية التاجية عند إستعمالها فى جرعات مهدئة.