Fentanyl Antagonizes Diazepam on Carotid Sinus Baroreflex Control of Circulation in Rabbits

Masakatsu SAKAMOTO, Hisatoshi OHSUMI*, Takeshi SUMIDA*, Fukuichiro OKUMURA** and Tohru MORIOKA

To investigate the effects of a combination of fentanyl and diazepam on carotid sinus baroreflex in conscious rabbits, we examined the responses of mean systemic arterial pressure (MAP), heart rate (HR) and total peripheral resistance (TPR) to bilateral carotid occlusion (BCO). Seven rabbits were given 0.5 mg·kg^{-1} of diazepam i.v. followed by 10 mcg·kg^{-1} of fentanyl i.v. at 5 min intervals (group 1), and the drugs were given in the reverse order to 5 other rabbits (group 2). BCO was repeated in conscious state (control) and after each drug injection. MAP responses did not differ from control response in either group when both drugs were given. In group 1, however, diazepam decreased HR response to 71.4% of control, and increased TPR response by 36%. Fentanyl administration reversed diazepam-induced changes in BCO responses to the control level. In group 2, fentanyl decreased TPR response to 61.6% of control and increased HR response by 41.5%. Administration of diazepam following fentanyl restored HR and TPR responses to control levels. Carotid sinus baroreflex gain was 3.1 ± 0.4 (mean ± SEM) in control and 3.1 ± 0.4 after administration of both drugs in 12 rabbits. The results suggest that a sedative dose of either fentanyl or diazepam antagonizes the other drug's action on the carotid sinus baroreflex. The combination of fentanyl and diazepam has little influence on carotid sinus baroreflex control of the circulation in rabbits. (Key words: carotid occlusion, drug interaction, arterial baroreflex)

(Sakamoto M, Ohsumi H, Sumida T, et al.: Fentanyl antagonizes diazepam on carotid sinus baroreflex control of circulation in rabbits. J Anesth 7: 210–217, 1993)

Department of Anesthesiology, Kumamoto University School of Medicine, Kumamoto, Japan

**Department of Anesthesiology, National Cardiovascular Center, Osaka, Japan*

***Department of Anesthesiology, School of Medicine, Yokohama City University, Yokohama, Japan*

Address reprint requests to Dr. Sakamoto: Department of Anesthesiology, Kumamoto University School of Medicine 1-1-1 Honjo, Kumamoto, 862 Japan

Opioid and benzodiazepine receptors in the central nervous system are involved in analgesia and hypnosis. They also have significant influences on neural regulation of arterial pressure and heart rate[1,2]. In previous reports[3,4], we showed that, in rabbits, when carotid sinus pressures were decreased by bilateral carotid occlusion (BCO), either a sedative dose of opioid (fentanyl) or benzodiazepine (diazepam) had op-

posing effects on carotid sinus baroreflex control of peripheral vascular resistance and heart rate. Diazepam attenuated BCO-induced tachycardia whereas it enhanced the reflex vasoconstriction response. By contrast, fentanyl attenuated the vasoconstrictor respnse and augmented reflex tachycardia induced by BCO. In addition to the contrasting effects on the arterial baroreflex, several investigators have reported central interaction of opiates (morphine, fentanyl) and benzodiazepine derivatives (diazepam, midazolam) on nociception through gamma aminobutyric acid (GABA) system[5,6]. GABA system is also involved in the baroreflex control of circulation. However, it is not well known how the combination of the two drugs modifies arterial baroreflex function. Kotrly, et al.[7] observed that the combination of such drugs with nitrous oxide attenuated baroreflex-mediated tachycardia in humans. However, there have been no reports on effects of the combination of fentanyl and diazepam on the reflex control of arterial pressure and peripheral circulation.

The present study was performed to investigate how the combination of fentanyl and diazepam modified carotid sinus baroreflex control of hemodynamics. Carotid sinus baroreflex was assessed by bilateral carotid occlusion (BCO). The degree of carotid occlusion was adjusted to hold carotid sinus pressure at −15 to −20 mmHg from the base line pressure. To study the effects of fentanyl and/or diazepam on carotid sinus baroreflex control of circulation, we measured arterial pressure and ascending aortic flow, as well as heart rate, and calculated total peripheral resistance.

Materials and Methods

Surgical preparation

Twelve rabbits (2.8–3.4 kg) were used in this study. All anesthetic and surgical procedures were performed in compliance with institutional guidelines. Anesthesia was induced with pentobarbital sodium (30 $mg \cdot kg^{-1}$ i.v.) and the level of anesthesia was maintained with supplemental dose of pentobarbital and inhalation of 60% nitrous oxide in oxygen. Surgical interventions were divided into two stages to minimize surgical stress for the animals.

In the first surgical procedure, the common carotid arteries were exposed, and an occlusion cuff was placed around each of them 1–2 cm caudal to the carotid sinus. The tubing for the occluders was routed subcutaneously to the dorsal side of the neck and fixed to the skin at the exit point. The aortic nerves were separated from the surrounding tissues and transected at the mid cervical level to interrupt the afferent pathways from the aortic baroreceptors. An electromagnetic flow probe (FC-060TS, Nihonkohden) was implanted through a right thoracotomy on the root of the asecnding aorta for measurement of ascending aortic flow. The probe cable was exteriorized at the back through a subcutaneous tunnel. The animals were allowed 7–10 days to recover from the surgical stress.

The second surgery was performed to implant arterial catheters. Under pentobarbital anesthesia, a catheter was inserted into the iliac artery via the femoral artery for measurement of systemic arterial pressure. The catheter was exteriorized through a skin button at the mid lumbar level of the back. Another catheter was implanted into the external carotid artery via the mandibular artery. The tip of this catheter was placed 1–2 cm distal to the carotid sinus. The catheter was passed subcutaneously and fixed in the neck at the exit point. Each catheter was flushed with 1 ml of saline containing 1000 units of heparin. Correct

Table 1. Baseline hemodynamics before and after the drug administration

Group 1. (n=7)

	MAP	HR	MAF	TPR
Control	86 ± 4	258 ± 15	568 ± 38	.143 ± .025
Diazepam	91 ± 4	261 ± 10	490 ± 38⋆	.161 ± .008⋆
Diazepam^{+} Fentanyl	87 ± 5	229 ± 16⋆	487 ± 34⋆	.163 ± .010⋆

Group 2. (n=5)

	MAP	HR	MAF	TPR
Control	85 ± 3	250 ± 10	556 ± 29	.153 ± .004
Fentanyl	85 ± 2	204 ± 17⋆	465 ± 28⋆	.184 ± .014⋆
Fentanyl^{+} Diazepam	82 ± 3	242 ± 10	546 ± 28	.151 ± .004

Shown baseline hemodynamic changes after 10 mcg·kg^{-1} of fentanyl or/and 0.5 mg·kg^{-1} of diazepam in group 1 and group 2.

Values are mean ± SEM. ⋆ indicates $P < 0.05$ compared with control

MAP; mean systemic arterial pressure (mmHg), HR; heart rate (beat·min^{-1}), MAF; mean ascending aortic flow (ml·min^{-1}), TPR; total peripheral resistance (mmHg·min·ml^{-1})

placement of the arterial catheter was confirmed after the end of experiment.

Experimental protocol

The experiment was performed in conscious rabbits 2 days after the last surgery. The carotid sinus baroreflex was tested while the rabbits sat quietly in a small box. After the experimental setup was completed, at least one hour was allowed for stabilization of the rabbits' hemodynamics. To examine the effects of fentanyl and diazepam on the carotid sinus baroreflex control of circulation, BCO was done under the following conditions in seven rabbits (group 1), 1) in conscious state (control), 2) after the administration of 0.5 mg·kg^{-1} of diazepam, and 3) after the administration of 10 mcg·kg^{-1} of fentanyl following the administration of diazepam. In the remaining five rabbits (group 2), fentanyl and diazepam were administered in the reverse order. The drugs were given via an ear vein at 5 min intervals. The doses of both drugs were determined as the amount which had caused significant alterations in the arterial baroreflex function in the previous studies[3,4]. Arterial blood samples for blood gas analysis were taken within 1–3 min after each experiment.

Bilateral carotid occlusion

Both common carotid arteries were occluded simultaneously for 1 min. Carotid occluders were inflated to steadily reduce the mean carotid sinus pressure by 15 to 20 mmHg under each condition. Hemodynamic parameters were continuously recorded before and during BCO, and the hemodynamic responses to BCO were obtained at 30–60 sec. Instantaneous and mean systemic arterial pressures, instantaneous carotid sinus pressure (CSP), and mean ascending aortic flow (MAF) were recorded. Total peripheral resistance (TPR) was calculated as the ratio of mean systemic arterial pressure (MAP) to MAF. To evaluate the hemodynamic responses to BCO, we obtained the changes in each hemodynamic vaiable from base line values

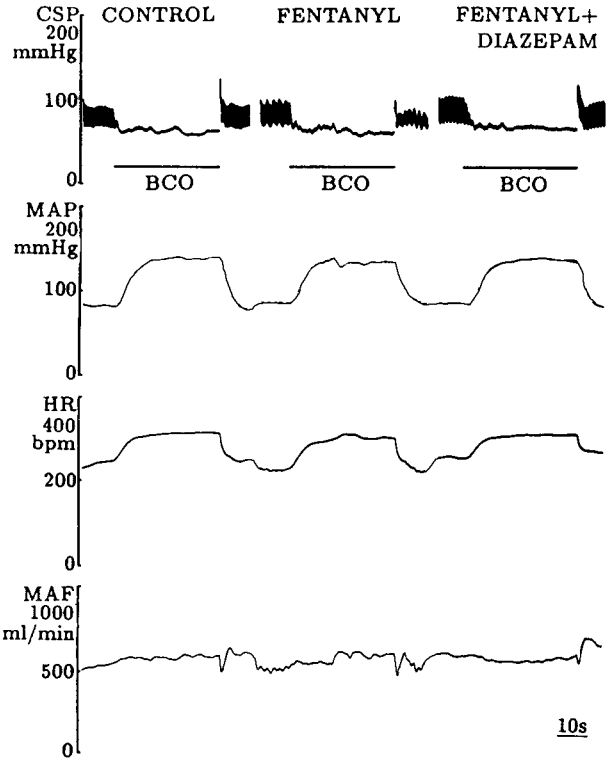

Fig. 1. Hemodynamic variables during bilateral carotid occlusion in control state, after 10 mcg·kg^{-1} of fentanyl, and 0.5 mg·kg^{-1} of diazepam administration following fentanyl in one rabbit. CSP; carotid sinus pressure, MAP; mean systemic arterial pressure, HR; heart rate, MAF; mean ascending aortic flow

during BCO. We also quantified the effects of the drugs on the carotid sinus baroreflex function by determining the reflex gain, which was expressed as a ratio of increase in MAP to fall in CSP (ΔMAP/ΔCSP). Arterial pressure was measured with a Statham P23 ID transducer. The ascending aortic flow was derived continuously by an electromagnetic flowmeter (MPV-2100, Nihonkohden). The level of instantaneous aortic flow during diastole was assumed to be zero flow and mean aortic flow was derived from instantaneous aortic flow by a low pass filter (time constant, 1 second). HR was measured using a cardiotachometer (1321, Nihondenki-Sanei) triggered by the ascending aortic flow signal.

Statistical analysis Values are reported as means ± SEM. Student's t-test for paired data or one way analysis of variance with repeated measures and a post-hoc Dunnett's test were applied to analyze effects of fentanyl and/or diazepam on the BCO responses. A $P < 0.05$ was considered statistically significant.

Results

After administration of fentanyl and diazepam, all 12 rabbits became calm and quiet. Their respiratory rates were decreased, and arterial P_{CO_2} was elevated significantly from 31.9 ± 1.2 mmHg to 34.8 ± 1.5 mmHg; arterial P_{O_2} was reduced from, 87.9 ± 1.9 mmHg to 76.3 ± 2.3 mmHg. Table 1 shows base line hemodyanamic values at control and after each drug adminstration in both group 1 and group 2. Neither drug affected base line MAP. In group 1, diazepam reduced MAF and increased TPR. In group 2, fentanyl reduced HR and MAF.

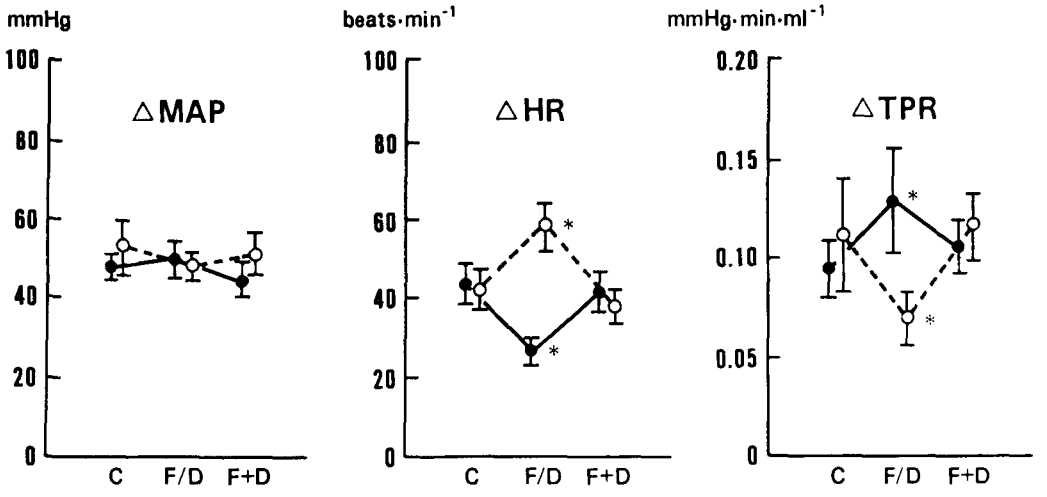

Fig. 2. Hemodynamic responses to bilateral carotid occlusion (BCO) in group 1 (closed circle) and group 2 (open circle). C; control, F; fentanyl, D; diazepam, F/D; after administration of a first drug (that is, 0.5 mg·kg^{-1} diazepam in group 1 or 10 mcg·kg^{-1} of fentanyl in group 2), F+D; after both 10 mcg·kg^{-1} of fentanyl and 0.5 mg·kg^{-1} of diazepam.

ΔMAP; systemic mean arterial pressure responses to BCO, ΔHR; heart rate response to BCO, ΔTPR; total peripheral resistance response to BCO, ΔMAF; mean aortic flow response to BCO. Values are means ± SEM.

* indicates $P < 0.05$ as compared with control.

Figure 1 shows a representative record of CSP, MAP, HR and MAF responses to BCO in one rabbit. Shown from the left to the right are control, after 10 mdg·kg^{-1} of fentanyl and after 0.5 mg·kg^{-1} of diazepam following fentanyl. BCO increased MAP, HR and TPR under all three conditions. Fugure 2 shows MAP, HR and TPR responses to BCO in both groups. Administration of fentanyl or diazepam caused significant changes in HR and TPR responses to BCO. In group 1, diazepam significantly increased TPR response and reduced HR response. However, fentanyl administered following diazepam reversed each response to control level. MAP response was not affected by either diazepam or fentanyl. In group 2, on the other hand, fentanyl significantly attenuated TPR response and increased HR responses. All hemodynamic responses to BCO were restored to control values after the administration of diazepam following fentanyl.

Figure 3 shows the hemodynamic responses to BCO at control and after the administraion of fentanyl 10 mcg·kg^{-1} and diazepam 0.5 mg·kg^{-1} in all 12 rabbits. During BCO, CSP was reduced by 17 ± 1 mmHg in control and by 16 ± 1 mmHg after the drug administraion. There was no statistically significant difference between these values. The combination of the drugs did not change any of the hemodynamic responses to BCO. The reflex gain was 3.1 ± 0.4 under control conditions and 3.1 ± 0.4 after the drug administraion. Thus, the combination of 10 mcg·kg^{-1} of fentanyl and 0.5 mg·kg^{-1} of diazepam did not have a significant influence on the carotid sinus baroreflex gain.

Discussion

In the present study, we showed that the combination of fentanyl and diazepam had little influence on carotid sinus baroreflex control of hemodynam-

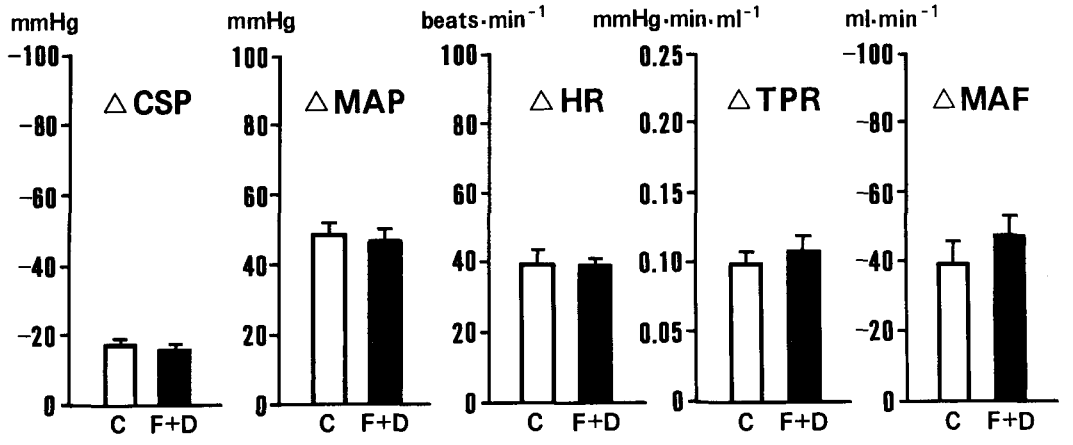

Fig. 3. Hemodyanmic responses to bilateral carotid occlusion (BCO) in control (C) and after both 10 mcg·kg^{-1} of fentanyl and 0.5 mg·kg^{-1} of diazepam (F+D) in all 12 rabbits (group 1 and group 2). ΔCSP; decrease in carotid sinus pressure during BCO. Values are means ± SEM. There were no statistical differences between control and after drug administration.

ics in conscious rabbits. However, the administration of either fentanyl or diazepam alone caused significant alterations in the baroreflex control of HR and TPR as shown in previous studies[3,4]. The second drug administered reversed the changes in the hemodynamic response to BCO induced by the drug given first. After the administration of both drugs, the hemodynamic responses to BCO were not significantly attenuated as compared with those in the conscious state.

Only few studies have focused on the effects of fentanyl-diazepam on baroreflex function. Kotrly et al. documented the influences of fentanyl and diazepam with nitrous oxide on arterial baroreflex control of HR in humans[7]. They showd that the comination of more than two anesthetics decreased the reflex tachycardia induced by sodium nitroprusside infusion whereas the HR responses to phenylephrine infusion or the neck suction were preserved. Their result regarding the reflex control of HR is not consistent with ours. This difference may be due to the method used to elicit the baroreflex, or to superimposed anesthetics (nitrous oxide), or species difference. There have been no report on the influence of fentanyl and diazepam on arterial baroreflex control of arterial pressure and peripheral vascular resistance. Ebert et al. documented the effects of fentanyl and diazepam on the cardiopulmonary baroreflex control of peripheral circulation[8]. They showed that fentanyl and diazepam did not alter cardiopulmonary baroreflex-induced vasoconstriction in humans. In the present study, we showed that the combination of fentanyl and diazepam did not change carotid sinus baroreflex control of circulation, neither HR, MAP nor TPR. Furthermore, the carotid sinus baroreflex gain was not changed after administration of these drugs.

The rabbits were only sedated with the amount of fentanyl or diazepam given in this study. However, these doses were sufficient to change the responses to BCO. In a preliminary experiment, we had tried larger doses of the drugs. However, we could not continue that experiment further due to the respiratory depression. The administration of fentanyl and diazepam decreased respiratory rates, increased ar-

terial P_{CO_2} and decreased arterial P_{O_2} in this study. In the previous study[3], we produced the blood gas changes similar to the above by inhalation of hypercarbic and hypoxic gas. Such small changes in arterial blood gas data did not exert significant influence on responses to BCO.

We reported that carotid sinus baroreflex control of arterial pressure was not changed by either fentanyl or diazepam in the previous studies[3,4]. However, fentanyl reduced BCO-induced vasoconstriction and augmented the tachycardia response. In contrast, diazepam enhanced vasoconstrictor responses to BCO and decreased HR response. We speculated that these influences on the reflex control of circulation could reflect the effects of each drug on autonomic nervous system. Namely, fentanyl has vagotonic and sympatholytic action[1,9,10] and diazepam has vagolytic action[2,11]. In the present study, we found antagonism between fentanyl and diazepam on the reflex control of circulation. Each drug reversed the changes in BCO responses induced by the drug given first. Kotrly et al.[7] showed similar interaction of diazepam and fentanyl on the arterial baroreflex control of HR. They observed that a high dose of fentanyl reversed the diazepam-induced depression of HR response when baroreflex was provoked by neck suction or phenylephrine infusion. They speculated that the vagotonic effect of fentanyl at higher doses was sufficient to overcome the depressant effect of diazepam.

The mechanism of fentanyl-diazepam interaction on the baroreflex function is not clear. Fentanyl is well known to exert its action mainly through opiate receptors. Diazepam binds to benzodiazepine receptors to facilitate GABA transmission in the central nervous system. Several investigators have documented that benzodiazepines antagonize the opiate-induced analgesia[5,6,12,13] and that the GABA system in the central nervous system sppears to be involved in the antagonism between opiates and benzodiazepines[5,6]. Further, morphine has been shown to have an affinity for GABA receptor ionophore complex, which causes blockade of GABA receptors[5,14–16]. This evidence suggests that antagonism between diazepam and fentanyl on the carotid sinus baroreflex function might occur through the GABA system. Alternatively, fentanyl and diazepam may exert their action separately on the autonomic nervous system, i.e., vagotonic and sympatholytic action of fentanyl may be thus exerted, and they might neutralize their influence on the autonomic nervous system.

In conclusion, we showed that fentanyl and diazepam had antagonistic effects on carotid sinus baroreflex control of circulation in conscious rabbits. Although either drug alone altered the reflex control of circulation, the drug given second antagonized the changes induced by the drug given first. Consequently, the combination of 10 $mcg \cdot kg^{-1}$ of fentanyl and 0.5 $mg \cdot kg^{-1}$ of diazepam did not affect the carotid sinus baroreflex function.

(Received Jun. 3, 1992, accepted for publication Aug. 26, 1992)

References

1. Laubie M, Schmitt H: Site of the vagally mediated bradycardia induced by morphine-like agents and opioid peptides. Central Nervous System Mechanism in Hypertension. Perspectives in Cardiovascular Research. Edited by Buckly JP, Ferrario CM, New York, Raven, vol 6, 1981, pp. 235–247
2. Hockman CH, Livingston KE: Inhibition of reflex vagal bradycardia by diazepam. Neuropharmacology 10:307–314, 1971
3. Ohsumi H, Sakamoto M, Yamazaki T, et al: Effects of fentanyl on carotid sinus baroreflex control of circulation

in rabbits. Am J Physiol 256:R625–R631, 1989
4. Sakamoto M, Ohsumi H, Yamazaki T, et al: Effects of diazepam on carotid sinus baroreflex control of circulation in rabbits. Acta Physiol Scand 139:281–287, 1990
5. Sivam S, Ho IK: Influence of morphine dependence on GABA-stimulated benzodiazepine binding to mouse brain synaptic membranes. Eur J Pharmacol 79:335–336, 1982
6. Zambotti F, Zonta N, Tammiso R, et al: Reversal of the effect of centrally-administered diazepam on morphine antinociception by specific (Ro 15–1788 and Ro 15–3505) and non-specific (bicuculline and caffeine) benzodiazepine antagonists. Naunyn-Schmiedeberg's Arch Pharmacol 333:43–46, 1986
7. Kotrly KJ, Ebert TJ, Vucins EJ, et al: Effects of fentanyl-diazepam-nitrous oxide anaesthesia on arterial baroreflex control of heart rate in man. Br J Anaesth 58:406–414, 1986
8. Ebert TJ, Kotrly KJ, Madsen KE, et al: Fentanyl-diazepam anesthesia with or without N_2O does not attenuate cardiopulmonary baroreflex mediated vasoconstrictor responses to controlled hypovolemia in humans. Anesth Analg 67:548–554, 1988
9. Daskalopoulos NTH, Laubie M. Schmitt H: Localization of the central sympatho-inhibitory effect of a narcotic analgesic agent, fentanyl in cats. Eur J Pharmacol 33:91–97, 1975
10. Franz DN, Hare BD, McCloskey KL: Spinal sympathetic nurons: possible sites of opiate-withdrawal suppression by clonidine. Science 215:1643–1645, 1982
11. Gerold M, Cavero I, Riggenbach H, et al: Analysis of cardiac dhronotropic responses to diazepam in conscious trained dogs. Eur J Pharmacol 35:361–368, 1976
12. Kissin I, Brown PT, Bradley Jr EL: Morphine and fentanyl anesthetic interactions with diazepam: Relative antagonism in rats. Anesth Analg 71:236–241, 1990
13. Rosland JH, Hole K: 1, 4-Benzodiazepines antagonize opiate induced antinociception in mice. Anesth Analg 71:242–248, 1990
14. Dingledine R, Iversen LL, Breuker E: Naloxone as a GABA antagonist: Evidence from ionophoretic receptor binding and convulsant studies. Eur J Pharmacol 47:19–27, 1978
15. Ticku MK, Huffman R: The effect of acute and chronic morphine administration on GABA receptor binding. Eur J Pharmacol 68:97–106, 1980
16. Jacquet YF, Saederup E, Squire RF: Non-stereo-specific exitatory actions of morphine may be due to GABA-A receptor blockade. Eur J Pharmacol 138:285–288, 1987