*Pharmacology Biochemistry and Behavior*, Vol. 47, pp. 171–175, 1994
Printed in the U.S.A. All rights reserved.

0091-3057/94 $6.00 + .00
Copyright © 1993 Pergamon Press Ltd.

# Chronic Administration of an $\alpha_2$ Adrenergic Agonist Desensitizes Rats to the Anesthetic Effects of Dexmedetomidine

KRISTINA REID, YUKIO HAYASHI, TIAN-ZHI GUO, CHRISTIANE CORREA-SALES, CARLA NACIF-COELHO AND MERVYN MAZE[1]

*Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305 and VA Medical Center, 3801 Miranda Avenue, Palo Alto, CA 94304*

Received 15 December 1992

REID, K., Y. HAYASHI, T.-Z. GUO, C. CORREA-SALES, C. NACIF-COELHO AND M. MAZE. *Chronic administration of an $\alpha_2$ adrenergic agonist desensitizes rats to the anesthetic effects of dexmedetomidine.* PHARMACOL BIOCHEM BEHAV 47(1) 171–175, 1994. — $\alpha_2$ adrenergic agonists are being administered perioperatively to facilitate the anesthetic management of the surgical patient. In some clinical settings, use of $\alpha_2$ adrenergic agonists has been extended into the postoperative period to prolong the patients' sedative and stress-free state. We studied whether the administration of $\alpha_2$ adrenergic agonists over an extended period of time would result in "desensitization" to the central actions of $\alpha_2$ adrenergic agonists.

Male Sprague–Dawley rats were administered dexmedetomidine, a highly selective $\alpha_2$ adrenergic agonist, at rates varying between 1 and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ via a chronically implanted SC osmotic pump. Spontaneous locomotor activity, tested in an open-field box, was significantly lower in both 3- and 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ treatment groups but returned to normal by the second or sixth day, respectively. The hypnotic response to dexmedetomidine IP was decreased in the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ dose group from the second day, and by the fourth day in the 3-$\mu g \cdot kg^{-1} \cdot h^{-1}$ group. Recovery from the desensitized state was rapid and occurred on the third day after pump removal in the 3-$\mu g \cdot kg^{-1} \cdot h^{-1}$ group and by the fifth day after pump removal in the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ dose group. By using a higher dose of dexmedetomidine IP (250 $\mu g \cdot kg$ in lieu of 100 $\mu g/kg$) at day 7 in "tolerant" rats, the hypnotic response could partially be "restored" towards normal. An attenuated hypnotic response could still be demonstrated even when dexmedetomidine was administered directly into the locus coeruleus (LC) in rats pretreated chronically with dexmedetomidine. In no case could hypnotic responsiveness be restored to normal by pretreating with the $\alpha_1$ adrenergic antagonist prazosin. The minimum anesthetic concentration (MAC) for halothane was not altered in the "tolerant" rats.

These data indicate that hyporesponsiveness develops to the central depressant effects of an $\alpha_2$ adrenergic receptor agonist, through a pharmacodynamic mechanism.

Adrenergic receptor: $\alpha_2$, agonist  Dexmedetomidine  Tolerance  Desensitization  Locus coeruleus
Anesthesia: hypnosis

ADMINISTRATION of $\alpha_2$ agonists throughout the entire perioperative period has been advocated to maximize the beneficial effects of these compounds in the surgical patient. Thus, the preoperative sedative and anxiolytic effects, the intraoperative decrease in anesthetic requirements, and postoperative decrease in analgesic requirements are best achieved by a prolonged continuous administration (15). Such prolonged administration of $\alpha_2$ agonists is not unusual, since this class of agent has been used in the management of chronic hypertension. While the beneficial antihypertensive effect is sustained over a period of years, the unwanted side effects, such as sedation, are relatively short-lived (4). The waning sedative effect suggests that tolerance may develop to the central nervous system (CNS) depressant properties of the $\alpha_2$ agonists.

The development of tolerance is a widespread biologic process in which responsiveness decreases with continuing exposure. This has been particularly well characterized for responses mediated by adrenergic receptors, especially of the $\beta$ and $\alpha_1$ subclasses (17). However, much less is known for the $\alpha_2$ adrenoceptors. Therefore, in this study we sought to determine whether rats became desensitized to the CNS depressant properties of $\alpha_2$ agonists following prolonged administration.

[1] Requests for reprints should be addressed to Mervyn Maze, Anesthesiology Service (112A), PAVAMC, Miranda Avenue, Palo Alto, CA 94304.

## METHODS

### *Animal Preparation*

Male Sprague-Dawley rats weighing 220-250 g were chosen as the experimental model following approval of the experimental protocol from the Animal Care and Use Committee at the Palo Alto Veterans Administration Medical Center. The rats for the control and treatment groups originated from the same litter. Rats were stratified to match the distribution of the rats' weights as closely as possible in the control and treatment groups.

Rats were administered dexmedetomidine chronically using Alzet® osmotic minipumps (Model 2002, Alza, Palo Alto, CA) which discharge their contents at a mean pumping rate of $0.48 \pm 0.02\ \mu l \cdot h^{-1}$. The pumps were loaded to deliver one of four different doses of dexmedetomidine 1, 3, 5, or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for periods lasting from two to seven days, at which time the rats were tested for behavioral responses. The pumps were inserted SC during halothane anesthesia in the dorsal thoracic region. In some studies the pumps were removed after seven days to assess recovery. In the initial experiments control animals were also implanted with the osmotic pumps containing the vehicle only. This group did not differ in behavioral response from sham-operated control animals; therefore the latter were used.

A separate experiment was devised to investigate whether attenuation in the hypnotic/anesthetic response was due to a pharmacokinetic or pharmacodynamic mechanism. Rats were surgically prepared to inject the anesthetic test agent (see below) into the locus coeruleus (LC), the site of $\alpha_2$ anesthetic action (2). The left LC was stereotactically cannulated with a 24-g stainless steel cannula as previously described (2) according to the following coordinates: with the bregma as the reference, 1.2 mm lateral, 9.7 mm posterior, and at a depth of 6 mm from the skull. The surgical procedure was performed with the rat under halothane anesthesia, and the cannula was fixed in position with methylmethacrylate resin. In these stereotactically cannulated animals dexmedetomidine was delivered chronically SC as described earlier; after seven days, the hypnotic test agent (dexmedetomidine) was injected into the LC. Correct placement of the cannula at the superior border of the LC was confirmed histologically at the conclusion of the experiments.

### *Behavioral Testing*

*Spontaneous locomotor activity* of the animals was recorded with a motility meter (Animex, LKB, Bromma Sweden); the rat was placed in an open-field box on top of the activity meter, and motility, as defined by crossovers on a grid, was assessed for 2 min.

*Hypnotic response* to dexmedetomidine, IP, was defined by the loss of the rat's righting reflex (LORR), and its duration was measured in minutes and referred to as sleeptime. The duration of the loss of righting reflex was assessed as the time from the rat's inability to right itself when placed on its back until the time that it spontaneously reverted, completely, to the prone position. In experiments in which dexmedetomidine was administered into the LC, the number of animals losing their righting reflex was determined. All hypnotic/anesthetic testing was performed between 1000 and 1800, as described previously (6).

*Minimum anesthetic concentration (MAC) of halothane* was determined to investigate whether a change in anesthetic response to volatile anesthetic agents was induced by chronic dexmedetomidine treatment. MAC was determined as previously described (16).

### *Dexmedetomidine Plasma Levels*

In order to exclude a pharmacokinetic mechanism for changes in $\alpha_2$ adrenergic responsiveness, plasma was obtained for measurement of dexmedetomidine, after IP administration, by a radioreceptor assay, as previously described (13), at the time that the animals regained their righting reflex. If the rats did not lose their righting reflex, they were sacrificed 1 h after dexmedetomidine administration.

### *Statistical Analysis*

Data are reported as mean ± SE and were compared by analysis of variance (ANOVA) with post hoc Scheffe and Fischer test or chi square analysis where appropriate.

## RESULTS

Spontaneous locomotor activity was significantly lower in the medium (3 $\mu g$) and high (10 $\mu g$) dexmedetomidine dose treatment groups (Fig. 1). The activity returned to normal on the second day for the medium dose group, while this only occurred consistently by the seventh day in the high dose group.

The hypnotic/anesthetic response to dexmedetomidine, 100 $\mu g \cdot kg^{-1}$ IP, was attenuated in the chronically treated rats (Fig. 2A). To test whether the hypnotic responsiveness in the chronically treated rats could be restored, a higher dose of dexmedetomidine, 250 $\mu g \cdot kg^{-1}$ IP, was administered. While the hypnotic response was still significantly attenuated at this higher dose (Fig. 2B), loss of righting reflex did in fact occur. In the time-course experiments, attenuated anesthetic responsiveness to dexmedetomidine 100 $\mu g \cdot kg^{-1}$ was noted in the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ group by the second day (Fig. 3) and was present in both the 3- and the 10-$\mu g \cdot kg^{-1} \cdot h^{-1}$ groups by the

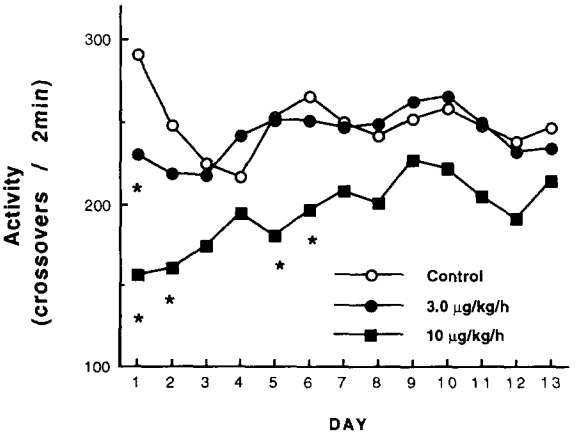


FIG. 1. Effect of chronic dexmedetomidine on spontaneous locomotor activity. Rats were implanted with osmotic pumps set to deliver 0 ($n$ = 8), 3 ($n$ = 9) or 10 ($n$ = 10) $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 13 days. Activity was measured in an open field by the number of crossovers in a grid as detected by photosensors. The same rats were monitored repeatedly on the specified days after pump implantation. Data were analyzed by analysis of variance and post hoc Scheffe's test where appropriate. *$p < 0.05$ compared to the control group for each day.

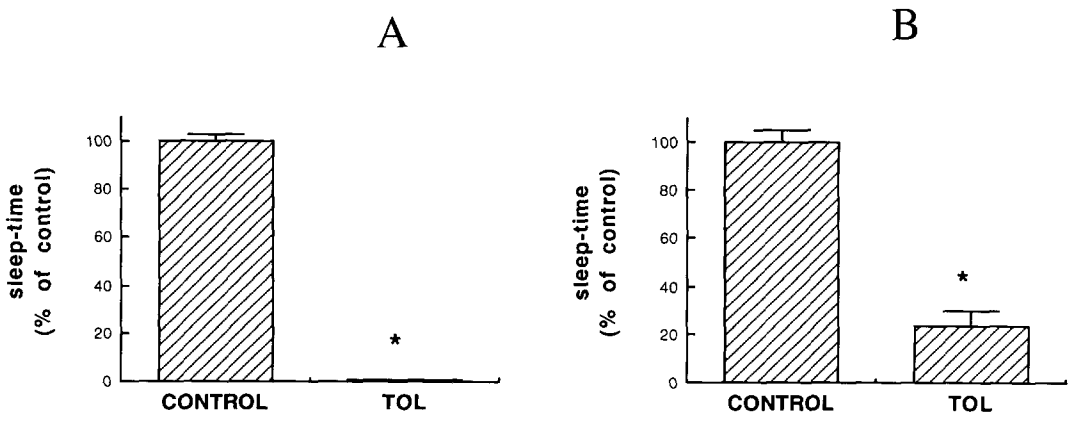


FIG. 2. (A) Hypnotic response to dexmedetomidine 100 $\mu g \cdot kg^{-1}$ IP in rats treated chronically with dexmedetomidine. Rats ($n$ = 10 per group) were implanted with osmotic pumps set to deliver 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (Tol) or sham-operated (Control). On the seventh day rats were administered dexmedetomidine 100 $\mu g \cdot kg^{-1}$ IP and the duration of loss of righting reflex was measured. Data are expressed with respect to the control group, which was considered to be 100% responsive. Data were analyzed by analysis of variance and post hoc Fisher test where appropriate. *$p < 0.05$. (B) Hypnotic response to dexmedetomidine 250 $\mu g \cdot kg^{-1}$ IP in rats treated chronically with dexmedetomidine. Rats ($n$ = 10 per group) were implanted with osmotic pumps set to deliver 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (Tol) or sham-operated (Control). On the seventh day rats were administered dexmedetomidine 250 $\mu g \cdot kg^{-1}$ IP and the duration of loss of righting reflex was measured. Data are expressed with respect to the control group, which was considered to be 100% responsive. Data were analyzed by analysis of variance (ANOVA) and post hoc Fisher test where appropriate. *$p < 0.05$.

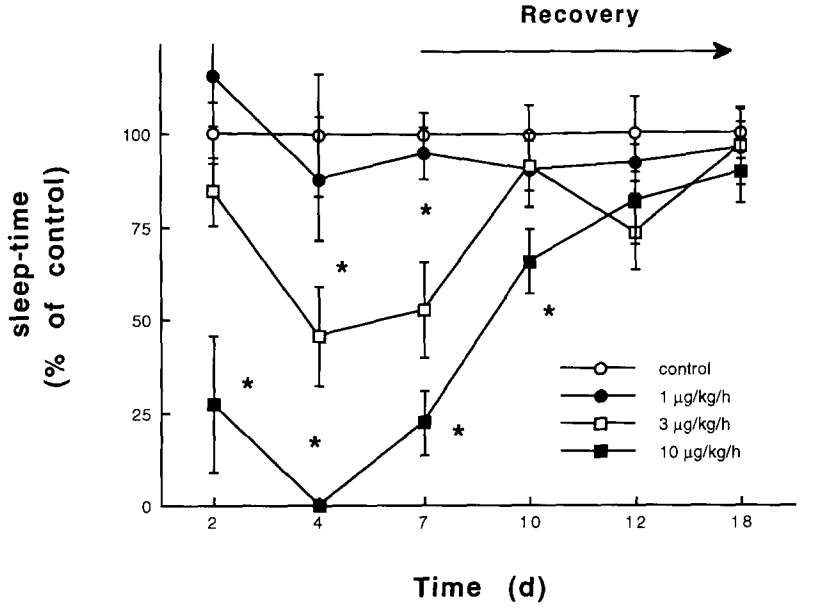


FIG. 3. Hypnotic response to dexmedetomidine 100 $\mu g \cdot kg^{-1}$ IP in rats administered dexmedetomidine for various times. Rats were implanted with osmotic pumps set to deliver 0, 1, 3, or 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$. At various times after implantation the hypnotic response to 100 $\mu g \cdot kg^{-1}$ IP was tested in separate cohorts of animals. In some animals, the pumps were removed after 7 days and the hypnotic response to 100 $\mu g \cdot kg^{-1}$ IP was tested at 3, 5, and 11 days later in separate cohorts. Data are expressed as a percentage of the response observed in the control group, which was considered to be 100% responsive. Data were analyzed by analysis of variance and post hoc Fisher test where appropriate. *$p < 0.05$. $n$ = 7–8 per group.

fourth day (Fig. 3). The low dose group (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) did not develop tolerance to the hypnotic effect of the $\alpha_2$ agonist. After seven days of treatment the pumps were removed, and the anesthetic responsiveness was restored to normal in the medium dose group by the third day, while in the high dose group this occurred five days after pump removal (Fig. 3).

Loss of righting reflex could not be induced with dexmedetomidine 7.0 $\mu g$ LC (an $ED_{95}$ dose) in rats pretreated chronically with dexmedetomidine (Fig. 4). However, when dexmedetomidine 70 $\mu g$ LC was administered (10 × the $ED_{95}$ dose), the hypnotic response was restored to normal (Fig. 4).

The MAC for halothane in the control rat population (1.05%) was not different from that observed in either the 3- (1.05%) or 10-mg $\cdot kg^{-1} \cdot h^{-1}$ (1.05%) groups which were treated for seven days.

The plasma dexmedetomidine levels were 13 ± 2.2 nM in naive rats and 21 ± 2.2 nM in chronically treated rats 1 h following dexmedetomidine administration.

Pretreatment with an $\alpha_1$ adrenergic antagonist, prazosin, did not restore the hypnotic response to normal (data not shown).

## DISCUSSION

These data suggest that rats become desensitized to the hypnotic/anesthetic action of an $\alpha_2$ agonist following chronic treatment with an $\alpha_2$ agonist. The desensitized state is due to a pharmacodynamic and not a pharmacokinetic mechanism, since the attenuated anesthetic response is evident even when the $\alpha_2$ agonist is delivered directly into the LC (Fig. 4), the site of $\alpha_2$ anesthetic action. The pharmacodynamic mechanism for this desensitization is not due to functional antagonism by an increase in $\alpha_1$ responsiveness (7), since the anesthetic response to systemically administered dexmedetomidine remains attenuated in the presence of prazosin, an $\alpha_1$ antagonist.

A desensitized state exists when the response to the agonist is attenuated or abbreviated. Tolerance is said to occur when a higher dose of agonist is required to produce the expected response. To determine whether the rats are indeed tolerant would require one to escalate the dose of $\alpha_2$ agonist further and establish that the hypnotic response can be restored. In the experiments in which a 2.5 times higher dose of dexmedetomidine was administered IP (Fig. 2B), responsiveness was still not completely restored. We did not administer higher doses of dexmedetomidine, since an $\alpha_1$ effect might become evident (14) which functionally antagonizes the $\alpha_2$ hypnotic response (7). An alternative approach was to perform a dose-response curve to an $\alpha_2$ agonist delivered directly into the LC, at which site $\alpha_1$ functional antagonism does not exist (2). The LC data (Fig. 4) indicate that at a 10 times higher dose of dexmedetomidine responsiveness was restored to normal in rats chronically treated with dexmedetomidine 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Therefore the present data do suggest that the desensitized animals have developed true tolerance to the hypnotic effects of an $\alpha_2$ agonist. In previous studies involving the chronic administration of $\alpha_2$ agonists intrathecally, tolerance to the analgesic effects of this class of compound developed over the same time course as was noted for the hypnotic response (9).

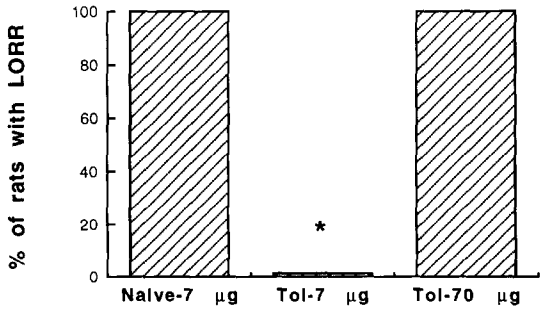


FIG. 4. Hypnotic response to dexmedetomidine in the locus coeruleus (LC) in rats administered dexmedetomidine for seven days. Rats were implanted with a cannula in the LC and simultaneously with an osmotic pump set to deliver either 0 (Naive) or 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ (Tol) for seven days. Rats were then tested for their hypnotic response to dexmedetomidine 7 or 70 $\mu g$ LC. Data are expressed as a percentage of rats with loss of the rat's righting reflex (LORR). Data were analyzed by chi square analysis. $*p < 0.05$. $n = 6$–9.

A desensitized state may be caused by pharmacokinetic mechanisms. Chronic administration of some drugs (e.g., phenobarbital) can induce its own biotransformation, resulting in a decreased drug concentration at the site of action. This does not appear to be the case for dexmedetomidine, since plasma concentrations of dexmedetomidine in the desensitized rats were significantly higher than the plasma levels in the naive rats when measured 1 h after drug administration. This higher concentration is probably due to the residual dexmedetomidine present in the pumps. Still, the hypnotic response was clearly attenuated in the chronically treated rats. We did not formally study the pharmacokinetic parameters of dexmedetomidine in the desensitized animals, and therefore we cannot define to what extent subtle pharmacokinetic alterations might have contributed to the desensitized state. However, the fact that the hypnotic response is absent when dexmedetomidine 7 $\mu g$ (an $ED_{95}$ dose) is introduced directly into the LC (Fig. 4) supports the contention that an alteration in end-organ responsiveness pertains.

It is notable that the response to halothane anesthesia was unaffected in the $\alpha_2$-desensitized rats. Although the precise molecular mechanisms which transduce the anesthetic action of volatile agents remain unknown, (12) there are no demonstrable molecular components which it shares with the $\alpha_2$ transduction mechanism apart from a possible common potassium conductance channel (3,5). We did not directly examine potassium conductance in the $\alpha_2$-desensitized animals.

If these animal data are extrapolative to the clinical paradigm, then there are likely to be several clinical scenarios in which altered responsiveness may be anticipated. Patients presenting for anesthesia and surgery may be on chronic treatment with $\alpha_2$ agonists for a variety of conditions, including hypertension (1) and spasticity (11). It is possible that the expected beneficial effects of anesthetic management of this class of agents may not obtain in such a patient population. In fact, the sedative properties of clonidine tend to diminish with increasing time of drug administration (4). Some have suggested that subacute treatment with $\alpha_2$ agonists over the extended perioperative period may be beneficial in patients with coronary artery disease (10) because the sympatholytic state protects the patient from "catecholamine surges" during the stressful perioperative period. Our study only examined anesthetic responsiveness, but others (18) have suggested that the expected sympatholytic consequences of $\alpha_2$ agonists do not occur following chronic treatment. Therefore the putative myocardial protective effects of the $\alpha_2$ agonists may also wane over a period of days.

In conclusion, chronic dexmedetomidine administration desensitizes rats to the hypnotic/anesthetic properties of $\alpha_2$ agonists through a decrease in end-organ responsiveness. The molecular mechanism underlying the hyporesponsiveness is under investigation.

ACKNOWLEDGEMENTS

The authors thank Dr. Risto Lammintausta for providing us with dexmedetomidine, Dr. Eva Gustafsson for her technical assistance, and Dr. Frances Davies, Dr. Patti Maguire, and Mr. Brad Rabin for helpful discussions.

## REFERENCES

1. Barnett, A. J.; Cantor, S. Observations on the hypotensive action of catapres (St 155) in man. Med. J. Aust. 1:87–91, 1968.
2. Correa-Sales, C.; Rabin, B. C.; Maze, M. A hypnotic response to dexmedetomidine, an $\alpha_2$ agonist, is mediated in the locus coeruleus in rats. Anesthesiology 76:948–952; 1992.
3. Doze, V. A.; Chen, B.-X.; Tinklenberg, J. A.; Maze, M. Pertusis toxin and 4-aminopyridine differentially affect the hypnotic-anesthetic action of dexmedetomidine and pentobarbital. Anesthesiology 73: 304–307; 1990.
4. Ferder, L.; Inserra, F.; Medina, F. Safety aspects of long-term antihypertensive therapy (10 years) with clonidine. J. Cardiovasc. Pharmacol. 10(Suppl. 12):S104–S108; 1987.
5. Franks, N. P.; Lieb, W. R. Volatile general anesthetics activate a novel neuronal $K^+$ current. Nature 333:662–664; 1988.
6. Guo, T.-Z.; Maze, B.; Maze, M. Attenuation of central alpha$_2$ adrenergic action in diabetic rats. Pharmacol. Biochem. Behav. 39:383–387; 1991.
7. Guo, T.-Z.; Tinklenberg, J.; Oliker, R.; Maze, M. Central $\alpha_1$ adrenoreceptor stimulation functionally antagonizes the hypnotic response to dexmedetomidine, an $\alpha_2$ adrenoreceptor agonist. Anesthesiology 75:252–256; 1991.
8. Koulu, M.; Pesonen, U.; Virtanen, R. Chronic dexmedetomidine, a selective $\alpha_2$-agonist, decreases serotonin but not noradrenaline turnover in rat brainstem nuclei. Eur. J. Pharmacol. 176:151–157; 1990.
9. Loomis, C. W.; Milne, B.; Crevenko, F. W. Determination of cross-tolerance in rat spinal cord using intrathecal infusion via sequential mini-osmotic pumps. Pharmacol. Biochem. Behav. 26: 131–139; 1987.
10. Mangano, D. T.; Siliciano, D.; Hollenberg, M.; Leung, J. M.; Browner, W. S.; Goehner, P.; Merrick, S.; Verrier, E. Postoperative myocardial ischemia: Therapeutic trials using intensive analgesia following surgery. Anesthesiology 76:432–353; 1992.
11. Mathias, C. J.; Luckitt, J.; Desai, P.; Baker, H.; El Masri, W.; Frankel, H. L. Pharmacodynamics and pharmacokinetics of the oral antispastic agent tizanidine in patients with spinal cord injury. J. Rehabil. Res. Dev. 26:9–16; 1989.
12. Morgan, P.; Sedensky, M.; Meneely, P. M. Multiple sites of action of volatile anesthetics in Caenorhabditis elegans. Proc. Natl. Acad. Sci. U. S. A. 87:2965–2969; 1990.
13. Salonen, M.; Maze, M. Implementation of a radioreceptor assay for dexmedetomidine. Pharmacol. Toxicol.; in press.
14. Schwinn, A.; Correa-Sales, C.; Page, S. O.; Maze, M. Functional effects of activation of $\alpha_1$ adrenergic receptors by dexmedetomidine: in vivo and in vitro studies. J. Pharmacol. Exp. Ther. 259: 1147–1152; 1991.
15. Segal, I. S.; Jarvis, D. J.; Duncan, S. R.; White, P. F.; Maze M. Clinical efficacy of transdermal clonidine during the perioperative period. Anesthesiology 74:220–225; 1991.
16. Segal, I. S.; Walton, J. K.; Irwin, I.; DeLanney, L. T.; Ricaurte, G. A.; Langston, J. W.; Maze, M. Modulating role of dopamine on anesthetic requirements. Eur. J. Pharmacol. 186:9–15; 1990.
17. Sibley, D. R.; Benovic, J. L.; Caron, M. G.; Lefkowitz, R. J. Regulation of transmembrane signaling by receptor phosphorylation. Cell 48:913–922; 1987.
18. Stevens, C. W.; Yaksh, T. L. Time course characteristics of tolerance development to continuously infused antinociceptive agents in rat spinal cord. J. Pharmacol. Exp. Ther. 251:216–223; 1989.