Laboratory Investigations

Dexmedetomidine-induced decrease in cerebral blood flow is attenuated by verapamil in rats: a laser Doppler study

Ferenc Bari PhD, Gyöngyi Horváth MD, György Benedek MD, PhD DSc

This study was performed to examine the changes in local cortical blood flow (CoBF) after simultaneous administration of an alpha$_2$ adrenergic agonist (dexmedetomidine) and a calcium channel antagonist (verapamil) to urethane-anaesthetized rats. Dexmedetomidine (100 $\mu g \cdot kg^{-1}$) given intraperitoneally alone resulted in decreases in mean arterial blood pressure (MABP) ($F[27,140] = 3.43$; $P < 0.01$) and CoBF ($F[27,140] = 4.22$; $P < 0.01$), whereas the heart rate (HR) was increased ($F[27,140] = 2.33$; $P < 0.01$). Verapamil (2.5 $mg \cdot kg^{-1}$) given subcutaneously reduced the MABP ($F[27,140] = 3.41$; $P < 0.01$), but the HR and CoBF were not changed. Combined administration of the drugs decreased MAPB ($F[27,140] = 5.37$; $P < 0.01$), with no changes in CoBF and HR. The present data indicate that the calcium channel antagonist verapamil did not potentiate the haemodynamic effects of dexmedetomidine in rats, but rather attenuated the effect of dexmedetomidine on CoBF. This favourable interaction suggests a potential therapeutic role of these agents in maintaining cardiovascular stability during surgical interventions.

Cette étude a été réalisée pour évaluer les changements du débit sanguin cortical (DSCo) après l'administration simultanée d'un agoniste alpha$_2$ adrénergique (dexmédétomidine) et d'un inhibiteur calcique (vérapamil) à des rat anesthésiés à l'uréthane. La dexmédétomidine seule (100 $\mu g \cdot kg^{-1}$) administrée par la voie péritonéale provoque une baisse de la tension artérielle moyenne (TAM) ($F[27,140] = 3,43$; $P < 0,01$) et du DSCo ($F[27,140] = 4,22$; $P < 0,01$), pendant que la fréquence cardiaque (Fc) augmente $F[27,140] = 2,33$; $P < 0,01$). Le vérapamil (2,5 kg^{-1}) sous-cutané provoque une baisse de la TAM ($F[27,140] = 3,41$; $P < 0,01$). L'administration combinée des deux médicaments diminue la TAM ($F[27,140] = 5,37$; $P < 0,01$), sans modifier le DSCo et la Fc. Ces données indiquent que l'inhibiteur calcique vérapamil n'augmente pas les effets hémodynamiques de la dexmédétomidine chez le rat, mais atténue l'effet de la dexmédétomidine sur le DSCo. Cette enteraction favorable suggère la possibilité d'une l'application thérapeutique de ces agents pour le maintien de la stabilité cardiovasculaire pendant la chirurgie.

Key words
BRAIN: blood flow;
BLOOD PRESSURE;
HEART RATE;
MEASUREMENT TECHNIQUES: laser Doppler flowmetry;
SYMPATHETIC NERVOUS SYSTEM: alpha$_2$-adrenergic agonist, dexmedetomidine;
PHARMACOLOGY: verapamil.

From the Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary.
Address correspondence to: Dr. Gyöngyi Horváth, Department of Physiology, Albert Szent-Györgyi Medical University, H-6720 Szeged, Dóm tér 10, Hungary.
Accepted for publication 9th May, 1993.

Alpha$_2$-adrenergic agonists, as novel anaesthetic agents, are currently undergoing wide-ranging investigation. Their effects are exerted by stimulating pre- and post-synaptic alpha$_2$-adrenergic receptors both centrally and peripherally (for an excellent review, see Maze 1992[1]).

Clonidine, a well-known alpha$_2$-adrenergic agonist, has been shown to reduce narcotic and volatile anaesthetic requirements during surgery, to reduce plasma catecholamine levels, to improve haemodynamic stability, and to display potent analgesic properties.[1-6]

Dexmedetomidine has been demonstrated to be a highly selective alpha$_2$-adrenergic agonist. In receptor binding experiments, its alpha$_2$/alpha$_1$ selectivity ratio was found to be 1620, compared with 220 for clonidine.[7,8] Similarly to clonidine, dexmedetomidine decreases the anaesthetic requirements and also produces sedation, anxiolysis and analgesia.[1,9-13] Dexmedetomidine has been shown to alter the cardiovascular functions via the peripheral and central nervous systems.[12] It reduces both the heart rate (HR) and the mean arterial blood pressure (MABP). A decrease in cerebral blood flow in dogs as a consequence of dexmedetomidine treatment has also been reported.[14,15]

Calcium channel blockers are widely used as antiarrythmics and antihypertensives in cardiovascular therapy. The action of these agents is attributed to a selective inhibition of the calcium influx into the cardiac and smooth muscle cells, which results in vascular smooth muscle relaxation and vasodilatation, inhibition of cardiac automaticity and conduction and a reduction of myocardial contractility.[16,17] Calcium channel blockers can be involved in pharmacological interactions when used concurrently with other drugs.

It has been reported that calcium antagonists increase the potency of various anaesthetic and analgesic drugs in animals and humans.[18-25] We have recently shown that verapamil, a widely used calcium channel blocker, potentiates the anaesthetic efficacy of dexmedetomidine, while the calcium channel activator BAY K 8644 decreases it.[26]

Interestingly, few studies have been published on the haemodynamic interactions of calcium channel blockers and alpha$_2$ agonists[27-29] Alpha$_2$-adrenoreceptor-mediated vasoconstriction is effectively antagonized by calcium channel blockers both *in vivo* and *in vitro*; this action is therefore primarily attributed to the facilitated influx of extracellular calcium ions.[28] Bloor has shown that nifedipine attenuates the haemodynamic changes induced by intravenous dexmedetomidine in isoflurane-anaesthetized dogs.[29]

The present investigation was designed to characterize further the possible haemodynamic and cerebral microcirculatory interactions between dexmedetomidine and verapamil in urethane anaesthetized rats.

Methods

Male Wistar rats ($n = 18$) weighing 250–300 g were used in the study, which had been approved by Animal Investigation Committee of Albert Szent-Györgyi Medical University. The animals were anaesthetized with intraperitoneally (*ip*) injected urethane (1.2 g · kg^{-1}). The trachea was cannulated to allow the animal to breathe spontaneously. The femoral artery was cannulated for continuous measurement of the MABP via a pressure transducer (Statham P23DB). Body temperature was kept at 37.0 ± 0.5°C by a heating pad. The HR was derived from the ECG.

Cortical blood flow (CoBF) was measured with a laser Doppler flowmeter (LDF): the PeriFlux PF3 (Perimed, Stockholm, Sweden). This technique measures the shift in frequency of laser light (632.8 nm, 2 mW) reflected from red cells moving along microvessels, and the number of moving red cells. This is considered to be a flux measurement. This device continually records the tissue perfusion, which allows an assessment of the dynamic responses of the local cortical microcirculation. The measurement volume is approximately 1 mm^3.[30-32] All measurements were performed with a frequency cut-off at 20 kHz and a time constant of 0.2 sec.

For placement of the LDF flow probe on the cortex, the heads of the rats were secured in a stereotaxic frame and a hole was drilled into the skull 2 mm lateral from the midline and just caudal to the coronal suture (cortical frontal 2 area).[33] After a stable flow signal had been obtained, the flow probe was kept in a fixed position on the cortex throughout the experiment. Since the LDF does not provide information from absolute values of CoBF the perfusion values are expressed as a percentage value of the baseline perfusion units.

Following the surgical procedure, the baseline MABP, HR and CoBF levels were recorded on a strip chart (Boveri-Brown, Austria) continuously for about one hour after drug administration.

Experimental protocol

Only rats with a MABP of ≥ 90 mmHg were used for the tests. The drugs were administered to three groups, with six animals in each group. The route of administration and doses were the same as described in our earlier experiments.[26]

In Group I, 100 μg · kg^{-1} dexmedetomidine was administered *ip* and saline subcutaneously (*sc*); in group II, 2.5 mg · kg^{-1} verapamil *sc* and saline *ip*; in group III, 100 μg · kg^{-1} dexmedetomidine *ip* and 2.5 mg · kg^{-1} verapamil *sc* simultaneously. The drugs were dissolved in saline.

Drugs

Dexmedetomidine.HCl was a generous gift from Farmos-Group Ltd. (Turku, Finland): verapamil.HCl was purchased from Orion (Helsinki, Finland).

TABLE Baseline levels of heart rate (HR), cortical blood flow (CoBF, as percentage of the mean value of a 5 min period prior to the drug administration) and mean arterial blood pressure (MABP) before drug administration in the treatment groups (mean ± SEM)

	DEX	*VER*	*DEX + VER*
HR (bpm)	207.0 ± 18.9	209.8 ± 9.3	204.6 ± 14.2
CoBF (%)	97.5 ± 1.9	102.7 ± 2.9	102.0 ± 3.7
MABP (mmHg)	107.5 ± 5.8	100.3 ± 5.3	108.7 ± 4.9

Statistics

Changes of variables over time were evaluated by one-way analysis of variance. Moreover, haemodynamic measurements were analysed for statistical significance by using two-way analysis of variance.[34] The main effects tested were drug treatment (group) and time. A probability level <0.05 was considered significant. The data are expressed as mean ± SEM.

In the result section F-values are given, and degrees of freedom indicated in parentheses together with the level of significance.

Results

There were no differences between the groups with respect to the baseline values of MABP, HR and CoBF (Table).

Figure 1 shows original recordings after administration of dexmedetomidine (A), verapamil (B), or the two drugs simultaneously (C). All the variables were stabilized in five to ten minutes after drug administration and a new steady state was achieved.

Intraperitoneal injection of dexmedetomidine at a dose of 100 $\mu g \cdot kg^{-1}$ resulted in decreases in MABP ($F[27,140] = 3.43$; $P < 0.01$) and CoBF ($F[27,140] = 4.22$; $P < 0.01$), whereas the HR was increased ($F[27,140] = 2.33$; $P < 0.01$).

Verapamil reduced the MABP ($F[27,140] = 3.41$; $P < 0.01$), but the HR and CoBF were not changed.

The combined drug administration of the drugs decreased MABP ($F[27,140] = 5.37$; $P < 0.01$), while the changes in CoBF and HR were not significant.

In Figure 2, the changes in HR after the different drug administrations are compared. The effects of the administration of the two drugs separately or in combination display differences with time ($F[2,445] = 18.0$; $P < 0.01$). Dexmedetomidine increased HR, while the combined drug administration diminished the tachycardic effect of the *ip* injection of dexmedetomidine.

In each group, the MABP decreased significantly relative to the baseline level (Figure 3), although the curves denoting the responses to different drug administrations displayed no significant differences ($F[2,501] = 2.3$). It

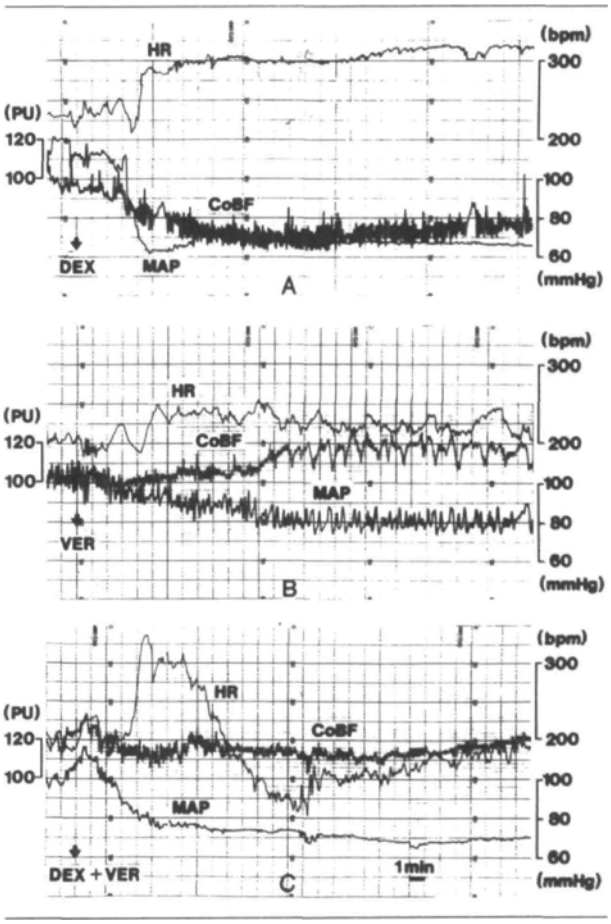

FIGURE 1 Representative recordings of physiological variables in rats after dexmedetomidine (A) (100 $\mu g \cdot kg^{-1}$; *ip*), verapamil (B) (2.5 $mg \cdot kg^{-1}$; *sc*) and simultaneous, combined dexmedetomidine (100 $\mu g \cdot kg^{-1}$; *ip*) and verapamil (2.5 $mg \cdot kg^{-1}$; *sc*) (C) administration. Abbreviations: CoBF - cortical blood flow, HR - heart rate, MAP - blood pressure, PU - perfusion unit (arbitrary units). Abscissa denotes time in minutes.

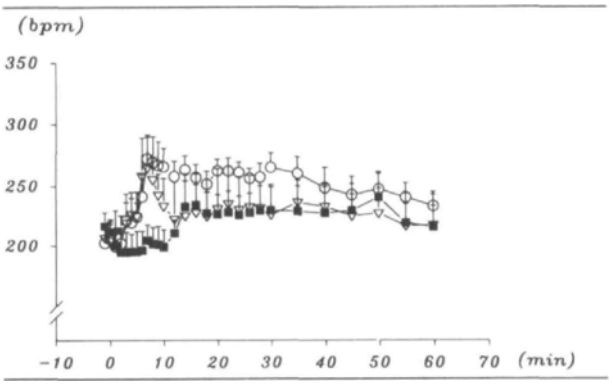

FIGURE 2 Changes in heart rate in rats after dexmedetomidine (○), verapamil (■) and simultaneous combined dexmedetomidine and verapamil (△) administration. Heart rate is expressed in beats per minute (bpm) units. Results are expressed as mean ± SEM.

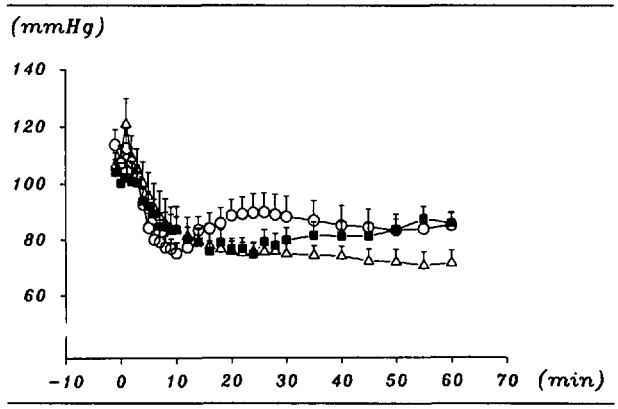

FIGURE 3 Changes in cortical blood flow after dexmedetomidine (○), verapamil (■) and simultaneous combined dexmedetomidine and verapamil (△) administration. Cortical blood flow is expressed as a percentage of baseline flow. Results are expressed as mean ± SEM.

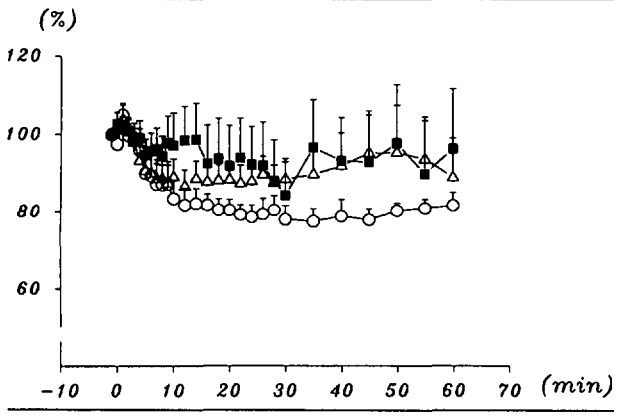

FIGURE 4 Changes in mean arterial blood pressure after dexmedetomidine (○), verapamil (■) and simultaneous combined dexmedetomidine and verapamil (△) administration. Results are expressed as mean ± SEM.

seems that the type of interaction was not of an additive pharmacodynamic nature.

There were differences between the effects on CoBF when dexmedetomidine and verapamil administered separately ($F[1,334] = 27.7$; $P < 0.01$). Differences were also found between the effect of dexmedetomidine and that of the co-administered drugs ($F[1,334] = 18.1$; $P < 0.01$), but not between those of verapamil and the binary combination (Figure 4).

Discussion

These results provide novel information with respect to the interaction between verapamil and dexmedetomidine on cardiovascular variables and cortical blood flow in rats. Our main finding was that a combined use of verapamil and dexmedetomidine reduced undesirable cardiovascular effects during anaesthesia. The hypotensive interaction of the two drugs is not additive; furthermore, verapamil attenuates the reduction of CoBF caused by dexmedetomidine administration.

The cardiovascular effects caused by systemic administration of alpha$_2$ agonists and calcium channel blockers are well documented.[1,16] The alpha$_2$ agonists exert powerful hypotensive and bradycardic effects. The site of action of these effects is considered predominantly to be in the rhombencephalon, and especially the nucleus tractus solitarius.[35,36] The mechanism whereby they elicit central cardiovascular effects has been related to the stimulation of central alpha$_2$-adrenoceptors, leading to reduced sympathetic drive that causes a decrease in blood pressure and bradycardia as well.[37,38] It is also apparent that alpha$_2$ agonists act more caudally via the spinal cord.[39] Furthermore, alpha$_2$ agonists reportedly decrease noradrenaline release at the sympathetic neuron terminals that is attributed to a presynaptic action.[3] Intravenous administration of alpha$_2$ agonists results in a transient increase in MABP due to peripheral vasoconstriction mediated by postsynaptic alpha$_2$-adrenoceptors on vascular smooth muscle. This action can be antagonized effectively by calcium channel blockers, and could therefore be in conjunction with the influx of extracellular calcium.[27,28]

We did not observe a bradycardic effect of *ip* injection of dexmedetomidine, but rather a tendency to tachycardia. This finding is compatible with other reports involving the same experimental methods.[40] There is recent evidence that anaesthesia induced by *ip* urethane reduces the bradycardic response consequent to administration of alpha$_2$-adrenoceptor agonists. The hypothesis has been advanced that urethane anaesthesia interferes with the prejunctional alpha-adrenoceptors located on cardiac sympathetic nerves.[41] In urethane-anaesthetized rats, the prejunctional alpha-adrenoreceptors could be maximally activated because of the elevated plasma levels of adrenaline (for a review see, Maggi[40]).

Since laser Doppler flowmetry is a continuous, fast-reacting and long-term stable method of blood flow measurement, it is suitable to detect blood flow changes in the cortical microvascular bed. The method is based on the direct detection of the velocity and number of red blood cells flowing through microvessels.[30,31] In our recent study, CoBF as well as systemic cardiovascular variables, were found to be stable during a long-lasting urethane anaesthesia.[32]

In line with the alpha$_2$ agonist effects detailed above, we observed a decrease in CoBF after dexmedetomidine administration to rats, similar to that described in dogs, and humans after clonidine.[14,15,42-44] The decrease in CoBF was probably due to an increase in cerebral vascular resistance, mediated through postsynaptic alpha$_2$-adrenoceptors.[45] Although the physiological role of sym-

pathetic innervations has been seriously doubted, it has been established that stimulation of the sympathetic nervous system by hypovolaemic hypotension may result in cerebral vasoconstriction via activation of alpha$_2$-adrenergic receptors.[46]

Calcium channel blockers are widely used in the treatment of hypertension and cardiac disturbances. The mechanism of MABP reduction produced by calcium channel blockers is usually ascribed to relaxation of the vascular smooth muscle, due to decreased entry of calcium into the cell via voltage-dependent channels.[47,48] Messing *et al.* have shown that small precapillary arterioles play an important role in the vasodilator action of calcium channel antagonists.[49] Thus, the antihypertensive effect of calcium antagonists may be based upon a diminution of vascular tone maintained by postsynaptic alpha$_2$-adrenoceptors.[49,50] It has recently been reported that calcium channel blockers decrease blood pressure after their administration into the nucleus of the solitary tract or into the intracerebroventricular space, indicating a central mechanism of action for calcium antagonists in blood pressure regulation.[51,52] Blockade of calcium channels in the heart results in negative chronotropic, dromotropic and inotropic effects.[16,53] Calcium channel blockers are also potent cerebral arterial dilators (Reves[54]). Cerebral arteries appear to be very sensitive to calcium antagonists, and a direct vasoconstrictor effect to calcium agonists has been observed in isolated cat and human cerebral arteries.[55,56]

Thus, it seems that calcium channel blockers and alpha$_2$ agonists have opposite effects on cerebral vessels. Calcium channel blockers induce vasodilatation, while alpha$_2$ agonists vasoconstriction, and these effects may be exerted directly on the cerebral blood vessels through alteration in calcium flux.

This consideration may explain our main finding that the calcium channel antagonist verapamil did not potentiate the haemodynamic effects of the alpha$_2$-adrenoceptor agonist, but rather attenuated the effect of dexmedetomidine on the CoBF. Our results are in line with earlier reports that calcium entry blockers attenuate the acute haemodynamic changes and reverse the coronary constrictor effect of dexmedetomidine.[29,57]

Acknowledgements

This work was supported by a grant (T 006379) from OTKA. The authors gratefully acknowledge the support of Farmos-Group Ltd for the supply of dexmedetomidine. The authors are very grateful to Dr. M. Szikszay for the constructive and helpful review of the manuscript. We thank Professor M. Bodosi (Szeged, Hungary) and Professor K. Pleschka (Bad Nauheim, Germany), for providing equipments for the experiments.

References

1 *Maze M, Tranquilli W.* Alpha-2 adrenoceptor agonists: defining the role in clinical anesthesia. Anesthesiology 1991; 74: 581–605.

2 *Ghignone M, Quintin L, Duke PC, Kehler CH, Calvillo O.* Effects of clonidine on narcotic requirements and hemodynamic response during induction of fentanyl anesthesia and endotracheal intubation. Anesthesiology 1986; 64: 36–42.

3 *Flacke JW, Bloor BC, Flacke WE, et al.* Reduced narcotic requirement by clonidine with improved hemodyamic and adrenergic stability in patients undergoing coronary bypass surgery. Anesthesiology 1987; 67: 11–9.

4 *Ghignone M, Calvillo O, Quintin L.* Anesthesia and hypertension: the effect of clonidine on perioperative hemodynamics and isoflurane requirements. Anesthesiology 1987; 67: 3–10.

5 *Ghignone M, Noe C, Calvillo O, Quintin L.* Anesthesia for opthalmic surgery in the elderly: the effects of clonidine on intraocular pressure, perioperative hemodynamics, and anesthetic requirement. Anesthesiology 1988; 68: 707–16.

6 *Flacke JW, Flacke WE, Bloor BC, Olewine S.* Effects of fentanyl, naloxone, and clonidine on hemodynamics and plasma catecholamine levels in dogs. Anesth Analg 1983; 62: 305–13.

7 *Virtanen R, Savola J-M, Saano V, Nyman L.* Characterization of the selectivity, specificity and potency of medetomidine as an α_2-adrenoceptor agonist. Eur J Pharmacol 1988; 150: 9–14.

8 *Scheinin H, Virtanen R, MacDonald E, Lammintausta R, Scheinin M.* Medetomidine–a novel α_2-adrenoceptor agonist: a review of its pharmacodynamic effects. Prog Neuropsychopharmacol Biol Psychiatry 1989; 13: 635–51.

9 *MacDonald E, Scheinin H, Scheinin M.* Behavioural and neurochemical effects of medetomidine, a novel veterinary sedative. Eur J Pharmacol 1988; 158: 119–27.

10 *Aantaa R, Kanto J, Scheinin M, Kallio A, Scheinin H.* Dexmedetomidine, an α_2-adrenoceptor agonist, reduces anesthetic requirements for patients undergoing minor gynecologic surgery. Anesthesiology 1990; 73: 230–5.

11 *Aantaa RE, Kanto JH, Scheinin M, Kallio AMI, Scheinin H.* Dexmedetomidine premedication for minor gynecologic surgery. Anaesth Analg 1990; 70: 407–13.

12 *Vickery RG, Sheridan BC, Segal IS, Maze M.* Anesthetic and hemodynamic effects of the stereoisomers of medetomidine, an α_2-adrenergic agonist, in halothane-anesthetized dogs. Anesth Analg 1988; 67: 611–5.

13 *Segal IS, Vickery RG, Walton JK, Doze VA, Maze M.* Dexmedetomidine dimishes halothane anesthetic requirements in rats through a postsynaptic alpha$_2$ adrenergic receptor. Anesthesiology 1988; 69: 818–23.

14 *Karlsson BR, Forsman M, Roald OK, Heier MS, Steen*

PA. Effect of dexmedetomidine, a selective and potent α_2-agonist, on cerebral blood flow and oxygen consumption during halothane anesthesia in dogs. Anesth Analg 1990; 71: 125–9.

15 *Zornow MH, Fleischer JE, Scheller MS, Nakakimura K, Drummond JC.* Dexmedetomidine, an α_2-adrenergic agonist, decreases cerebral blood flow in the isoflurane-anesthetized dog. Anesth Analg 1990; 70: 624–30.

16 *Fleckenstein-Grün G, Frey M, Fleckenstein A.* Calcium antagonists: mechanisms and therapeutic uses. TIPS 1984; 5: 283–7.

17 *Fleckenstein A.* Calcium antagonism in heart and vascular smooth muscle. Med Res Rev 1985; 5: 395–425.

18 *Dolin SJ, Little HJ.* Augmentation by calcium channel antagonists of general anaesthetic potency in mice. Br J Pharmacol 1986; 88: 909–14.

19 *Dolin SJ, Little HJ.* Effects of "nitrendipine" on nitrous oxide anesthesia, tolerance, and physical dependence. Anesthesiology 1989; 70: 91–7.

20 *Dolin SJ, Patch TL, Rabbani M, Taberner PV, Little HJ.* Differential interactions between benzodiazepines and the dihydropyridines, nitrendipine and BAY K 8644. Neuropharmacology 1991; 30: 217–24.

21 *Benedek G, Szikszay M.* Potentiation of thermoregulatory and analgesic effects of morphine by calcium antagonists. Pharmacol Res Commun 1984; 16: 1009–18.

22 *Horváth Gy, Benedek Gy, Szikszay M.* Enhancement of fentanyl analgesia by clonidine plus verapamil in rats. Anesth Analg 1990; 70: 284–8.

23 *Horváth Gy, Szikszay M, Benedek Gy.* Potentiated hypnotic action with a combination of fentanyl, a calcium channel blocker and an α_2-agonist in rats. Acta Anaesthesiol Scand 1992; 36: 170–4.

24 *von Bormann B, Boldt J, Sturm G, et al.* Calciumantagonisten in der Anaesthesie. Anaesthesist 1985; 34: 429–34.

25 *Godfraind T, Miller R, Wibo M.* Calcium antagonism and calcium entry blockade. Pharmacol Rev 1986; 38: 321–417.

26 *Horváth Gy, Szikszay M, Benedek Gy.* Calcium channels are involved in the hypnotic-anesthetic action of dexmedetomidine in rats. Anesth Analg 1992; 74: 884–8.

27 *Van Meel JCA, De Jonge A, Kalkman HO, Wilffert B, Timmermans PBMWM, Van Zwieten PA.* Vascular smooth muscle contraction initiated by postsynaptic α_2-adrenoceptor activation is induced by an influx of extracellular calcium. Eur J Pharmacol 1981; 69: 205–8.

28 *Timmermans PBMWM, Chiu AT, Thoolen MJMC.* Calcium handling in vasoconstriction to stimulation of $alpha_1$- and $alpha_2$-adrenoceptors. Can J Physiol Pharmacol 1986; 65: 1649–57.

29 *Bloor BC, Alper G, Frankland M, Flacke JW.* Nifedipine attenuates the acute hemodynamic changes that occur with intravenous dexmedetomidine in dogs. Anesth Analg 1989; 68: S321.

30 *Bonner R, Nossal R.* Model for laser Doppler measurements of blood flow in tissue. Applied Optics 1981; 20: 2097–107.

31 *Haberl RL, Heizer ML, Marmarou A, Ellis EF.* Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. Am J Physiol 1989; 256: H1247–H1254.

32 *Barzó P, Bari F, Dóczi T, Jancsó G, Bodosi M.* Significance of the rate of systemic change in blood pressure on short-term autoregulatory response in normotensive and spontaneously hypertensive rats. Neurosurgery 1993; 32: 611–8.

33 *Hebel R, Stromberg M.* Anatomy and Embryology of the Laboratory Rat. Worthsee: BioMed Verlag, 1986: 5.

34 *Kirkwood BR.* Essentials of Medical Statistics. Oxford: Blackwell; 1992.

35 *Unnerstall JR, Kopajtic TA, Kuhar MJ.* Distribution of α_2 agonist binding sites in the rat and human central nervous system: analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. Brain Res Rev 1984; 7: 69–101.

36 *Granata AR, Numao Y, Kumada M, Reis DJ.* A1 noradrenergic neurons tonically inhibit sympathoexcitatory neurons of the C1 area in rat brainstem. Brain Res 1986; 377: 127–46.

37 *Deckert V, Lachaud V, Parini A, Elghozi J-L.* Contribution of α_2-adrenoceptors to the central cardiovascular effects of clonidine and S 8350 in anesthetized rats. Clin Exp Pharmacol Physiol 1991; 18: 401–8.

38 *Isaac L.* Clonidine in the central nervous system: site and mechanism of hypotensive action. J Cardiovasc Pharmacol 1980; 2: S5–S19.

39 *Sannajust F, Barres C, Koenig-Berard E, Sassard J.* Central and peripheral effects of rilmenidine and clonidine in conscious and anaesthetized SHRs. Br J Pharmacol 1989; 96: 117.

40 *Maggi CA, Meli A.* Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: Cardiovascular system. Experientia 1986; 42: 292–6.

41 *Timmermans PVBWM, Fluitman PHM, Mac Kaay JCJ, Van Zwieten PH.* Hypotensive and bradycardic effects of classical sympathomimetic drugs upon intravenous administration. Archs Int Pharmacodyn Ther 1978; 231: 98–103.

42 *James IM, Larbi E, Zaimis E.* The effect of the acute intravenous administration of clonidine (St 155) on cerebral blood flow in man. Br J Pharmacol 1970; 39: 198P–199P.

43 *Kanawati IS, Yaksh TL, Anderson REE, Marsh RW.* Effects of clonidine on cerebral blood flow and response to arterial CO_2. J Cereb Blood Flow Metab 1986; 6: 358–65.

44 *Sherman GP, Grega GJ, Woods RJ.* Evidence for a central hypotensive mechanism of ST-155. Eur J Pharmacol 1968; 2: 326–8.

45 *Tsukahara T, Taniguchi T, Usui H, et al.* Sympathetic de-

nervation and alpha adrenoceptors in dog cerebral arteries. Arch Pharmacol 1986; 334: 436–43.

46 *Edvinsson L, MacKenzie ET, Robert J-P, Skärby T, Young AR.* Cerebrovascular responses to haemorrhagic hypotension in anaesthetized cats. Effects of α-adrenoceptor antagonists. Acta Physiol Scand 1985; 123: 317–23.

47 *Braunwald E.* Mechanism of action of calcium channel blocking agents. N Engl J Med 1982; 307: 1618–27.

48 *Vaghy PL, Willaims JS, Schwartz A.* Receptor pharmacology of calcium entry blocking agents. Am J Cardiol 1987; 59: 9A–17A.

49 *Messing M, Van Essen H, Smith TL, Smits JFM, Struyker-Boudier HAJ.* Microvascular actions of calcium channel antagonist. Eur J Pharmacol 1991; 198: 189–95.

50 *Faber JE.* In situ analysis of α-adrenoceptors on arteriolar and venular smooth muscle in rat skeletal muscle microcirculation. Circ Res 1988; 62: 37–50.

51 *Rabkin SW.* Diltiazem and verapamil lower blood pressure in the unanaesthetized rat though CNS mechanisms involving endogenous opioids. Clin Exp Pharmacol Physiol 1991; 18: 431–8.

52 *Higuchi S, Takeshita A, Ito N, Imaizumi T, Matsuguchi H, Nakamura M.* Arterial pressure and heart rate responses to calcium channel blockers administrated in the brainstem in rats. Circ Res 1985; 57: 244–51.

53 *Kawai C, Konishi T, Matsuyama E, Okazaki H.* Comparative effects of three calcium antagonists, diltiazem, verapamil and nifedipine, on the sinoatrial and atrioventricular nodes. Circulation 1981; 63: 1035–42.

54 *Reves JG, Kissin I, Lell WA, Tosone S.* Calcium entry blockers: uses and implications for anesthesiologists. Anesthesiology 1982; 57: 504–18.

55 *Allen GS, Bahr AL.* Cerebral arterial spasm. Part 10. Reversal of acute and chronic spasm in dogs with orally administered nifedipine. Neurosurgery 1979; 4: 43–9.

56 *Salaices M, Alonso MJ, Rico I, Fernández-Alfonso MS, Marin J.* Vasoconstrictor effect of the Ca^{2+} agonist Bay K 8644 on human cerebral arteries. Brain Res 1989; 490: 133–40.

57 *Flacke WE, Flacke JW, de Lange S, Lawrence CJ, Prinzen F.* Isradipine reverses coronary constrictor effects of dexmedetomidine. Anesthesiology 1990; 73: A608.