Influence of Transient Global Cerebral Ischemia on the Facilitatory Modulation of the Vagal Baroreflex in Dogs

Junichi Kurihara, Satoru Tamaoki and Hitoshi Kato

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

ABSTRACT—The influence of 5-min global cerebral ischemia on the facilitatory modulation of the vagal baroreflex through central α2-adrenoceptors or by the electrical stimulation of the septum was investigated in anesthetized dogs. Reflex bradycardia was produced by a bolus injection of phenylephrine at a dose which produces about a 25-mmHg increase in mean blood pressure. The ischemia was produced by the occlusion of the brachiocephalic and the left subclavian arteries with preceding ligation of the intercostal arteries. Clonidine at 10 μg, administered intracisternally, decreased the blood pressure and heart rate and facilitated the vagal reflex bradycardia. During the reperfusion period following ischemia, however, clonidine failed to affect the reflex bradycardia. Electrical stimulation of the septal region facilitated the reflex bradycardia without marked influences on the basal blood pressure and heart rate. The facilitatory effect was dependent on the frequency (10 to 75 Hz) and amplitude (3 to 15 V) of stimulation and was not observed after vagotomy or ischemic insult. These results suggest that 5-min global cerebral ischemia may produce the dysfunction of the neurons which are closely related to the baroreflex loop and receive the facilitatory modulation through α2-adrenoceptors and/or from the forebrain structures, leading to the dysfunction of the vagal baroreflex.

Keywords: Ischemia (cerebral), Baroreflex (vagal), α2-Adrenoceptor, Septum

Global cerebral ischemia of 5 min produces a selective dysfunction of the vagal component of the baroreflex in dogs (1). Since the pretreatments with α2-adrenoceptor blocking agents (2), but not MK-801 (3), a non-competitive N-methyl-D-aspartate (NMDA) antagonist, provided cerebral protection in this experimental model, it could be speculated that α2-adrenoceptors, but not NMDA receptors, might play a pathogenetical role. On the other hand, stimulation of the central α2-adrenoceptors has been shown to facilitate the vagal baroreflex (4–6). Thus, one of the hypotheses was that excessive activation of α2-adrenoceptors during ischemia and the early reperfusion period might alter the α2-adrenergic system, or injure the neurons with α2-adrenoceptors, leading to the dysfunction of neuronal networks that are essential to the vagal baroreflex and/or its modulatory systems.

In this context, we investigated firstly the influence of 5-min global cerebral ischemia on the effects of intracisternally administered clonidine to confirm the post-ischemic dysfunction of the central α2-adrenergic systems. Secondly, we attempted to investigate further the influence of ischemia on another neurogenic modulation of the baroreflex. For this purpose, we performed the electrical stimulation of the forebrain structure within the septum, which has been shown to selectively facilitate the vagal component of the baroreflex (7).

MATERIALS AND METHODS

Animals

Forty-eight mongrel dogs of either sex weighing 8 to 19 kg were anesthetized with sodium pentobarbital at 32 mg/kg, i.v., followed by an infusion of 3.2 mg/kg/hr, i.v. The animals were artificially ventilated and immobilized with suxamethonium chloride at 2 mg/kg, i.v., followed by an infusion of 1 mg/kg/hr, i.v. Arterial Po2, Pco2 and rectal temperature were maintained at about 100 mmHg, 35 mmHg and 38°C, respectively. Arterial blood pressure was measured from the left femoral artery by means of a pressure transducer (TP-200T; Nihon Kohden, Tokyo), and heart rate was measured by a heart rate counter (AT-600G, Nihon Kohden) triggered by the lead II ECG. The cortical EEG was continuously monitored from the parietal skull using a frequency analyzer (OEE-
Incomplete global cerebral ischemia was produced by a 5-min occlusion with clamps of the brachiocephalic artery and the left subclavian artery following ligations of about 14 intercostal arteries. In the first series of experiments, where clonidine was administered intracisternally, the rapid cessation of EEG following the ischemic insult was considered to represent a sufficient degree of cerebral blood flow reduction. In the second series of experiments, where the septum was electrically stimulated, the severity of ischemia was assessed by the reduction of the blood flow in the dorsal medulla oblongata measured by a tissue flow monitor (UMW-101; Unique Medical, Tokyo) using a plate-type thermocouple electrode. The mean residual blood flow during ischemia (8) in the latter experiments was 22.8±2.7% (n=6).

Stimulation of the septum

The head of animals (body weight of 12.3±1.2 kg, n=24) was fixed in a stereotaxic apparatus. According to the brain map by Lim et al. (9), a coaxial bipolar stimulating electrode was vertically inserted into the septum. The tip diameter of the electrode was 300 μm, and the cathode and anode were separated by about 1 mm. Electrical stimulation (1 msec, 10–75 Hz, 3–15 V) was applied by means of an electrical stimulator (SEN-7103, Nihon Kohden). The influence of stimulation on the baroreflex was assessed by comparing phenylephrine-induced reflex bradycardia before and during stimulation. Since only a small part of the septum possesses the ability to augment the baroreflex, we had to search for the proper site of stimulation, adjusting the location of the electrode tip. Practically, we fixed the electrode tip at the position where the stimulation (1 msec, 50 Hz, 10 V) produced 2- to 3-fold augmentation of reflex bradycardia. The final position of the tip of the electrode was 27.3±1.4 mm anterior and 14.3±1.7 mm vertical to the external auditory meatus and 1.8±0.1 mm lateral to the midline (n=24).

| Table 1. Influence of bilateral vagotomy on phenylephrine-induced reflex bradycardia and its facilitation by clonidine |
|---------------------------------------------------------------|
| **Drugs**                                                   |
| 1-Phenylephrine hydrochloride (Sigma, St. Louis, MO, USA) and suxamethonium chloride (Tokyo Kasei, Tokyo) were dissolved in physiological saline and administered into the cephalic vein. The dose of phenylephrine to produce about a 25-mmHg increase in mean arterial blood pressure was estimated in each animal. Clonidine hydrochloride (Sigma) at 1 mg/ml was also dissolved in saline, and 10 μl solution was injected with a microsyringe into the cisterna magna. The effect of clonidine on the vagal baroreflex was confirmed, in the prelimi- |
nary experiment, to reach a stable state within 30 min after administration.

Statistics
Comparisons between paired values were performed with the paired Student’s t-test. Multiple comparisons between mean values were performed with Bonferroni’s multiple-comparison test. Differences giving \( P < 0.05 \) were judged to be statistically significant.

RESULTS

Effects of clonidine on the vagal baroreflex
As shown in Table 1, phenylephrine-induced reflex bradycardia was significantly attenuated by the bilateral section of the cervical vagosympathetic trunk (vagotomy), but not its sham operation. Then the effect of 10 \( \mu \)g of clonidine on the baroreflex was assessed 30 min after intracisternal administration. Clonidine produced a significant increase in the extent of reflex bradycardia in animals subjected to sham vagotomy, while it failed to affect the reflex in vagotomized animals. These results indicate that the facilitatory effect of clonidine on reflex bradycardia is selective for the vagal component. In addition, the extents of hypotensive and bradycardic responses to clonidine in vagotomized dogs were significantly smaller than those in sham-vagotomized dogs: 24\( \pm \)6 vs. 50\( \pm \)5 mmHg decrease in mean blood pressure and 27\( \pm \)8 vs. 62\( \pm \)7 beats/min decrease in heart rate (n=6).

Influence of 5-min global cerebral ischemia on the effects of clonidine

In animals subjected to 5-min global cerebral ischemia, phenylephrine-induced reflex bradycardia at 60-min reperfusion was significantly smaller than that before ischemia, as shown in Table 2. Although subsequent intracisternal administration of clonidine at 10 \( \mu \)g decreased the arterial blood pressure and heart rate, the extents of changes were significantly smaller than those in sham-operated animals: 27\( \pm \)4 vs. 47\( \pm \)6 mmHg decrease in mean blood pressure and 11\( \pm \)2 vs. 41\( \pm \)3 beats/min decrease in heart rate (n=6). Furthermore, clonidine failed to produce a significant change in the reflex bradycardia during the reperfusion period, while it facilitated the reflex in sham-operated animals. These results indicate that 5-min global cerebral ischemia attenuates the central effects of clonidine on basal blood pressure and heart rate and abolishes the facilitatory effect on the vagal baroreflex.

| Table 2. Influence of 5-min global cerebral ischemia on phenylephrine-induced reflex bradycardia and its facilitation by clonidine |
|---------------------------------------------------------------------------------------------------------------|
|                                                                                                               |
| 5-min sham ischemia                                                                                          |
| Dose\(^a\) (\(\mu\)g/kg, i.v.)                                                                              | 6.2\( \pm \)1.1 | 6.5\( \pm \)1.1 | 6.5\( \pm \)1.1 |
| MBP (mmHg) before\(^b\)                                                                                     | 135\( \pm \)10  | 142\( \pm \)7  | 95\( \pm \)5**+/\# |
| \(\Delta\) MBP                                                                                               | 26\( \pm \)2   | 26\( \pm \)1   | 21\( \pm \)3   |
| PI (msec) before\(^b\)                                                                                      | 379\( \pm \)31 | 388\( \pm \)25 | 524\( \pm \)5\# |
| \(\Delta\) PI                                                                                               | 38\( \pm \)5   | 38\( \pm \)5   | 144\( \pm \)14**+/\# |
| \(\Delta\) PI/\(\Delta\) MBP (msec/mmHg)                                                                    | 1.46\( \pm \)0.21 | 1.45\( \pm \)0.19 | 7.03\( \pm \)0.53**+/\# |
| 5-min ischemia                                                                                               |
| Dose\(^a\) (\(\mu\)g/kg, i.v.)                                                                              | 4.3\( \pm \)0.4 | 10.2\( \pm \)0.7\# | 10.2\( \pm \)0.7\# |
| MBP (mmHg) before\(^b\)                                                                                     | 125\( \pm \)12  | 135\( \pm \)8  | 106\( \pm \)5  |
| \(\Delta\) MBP (mmHg)                                                                                        | 25\( \pm \)1   | 22\( \pm \)2   | 22\( \pm \)2   |
| PI (msec) before\(^b\)                                                                                      | 407\( \pm \)41  | 398\( \pm \)42  | 422\( \pm \)44  |
| \(\Delta\) PI (msec)                                                                                        | 37\( \pm \)4 | 16\( \pm \)3\# | 19\( \pm \)4\# |
| \(\Delta\) PI/\(\Delta\) MBP (msec/mmHg)                                                                    | 1.45\( \pm \)0.18 | 0.64\( \pm \)0.08**+/\# | 0.84\( \pm \)0.08**+/\# |

The results from 6 animals in each group are indicated as the mean \(\pm\) S.E.M. \(^a\)Dose of phenylephrine, \(^b\)value before phenylephrine, MBP: mean arterial blood pressure, PI: pulse interval. Phenylephrine was administered before ischemia or sham, 60 to 90 min after ischemia or sham, and 30 min after subsequent intracisternal administration of clonidine (10 \(\mu\)g). \(^*\)P < 0.05, \(^**\)P < 0.01: significantly different from the value before clonidine. \(^\#\)P < 0.05, \(#\)P < 0.01: significantly different from the value before ischemia or sham ischemia.
Facilitation of the vagal baroreflex by electrical stimulation of the septum

Figure 1 shows a typical example of the influence of the electrical stimulation (1 msec, 50 Hz, 10 V) of the septum. In the presence of the septal stimulation, phenylephrine-induced reflex bradycardia was clearly augmented. Following bilateral vagotomy, however, the same intensity of stimulation failed to augment the reflex bradycardia. The influence of the septal stimulation was dependent on the amplitude (3 to 15 V) and frequency (10 to 75 Hz) of the electrical stimulation, as shown in Fig. 2. Next, we used the submaximal condition of stimulation (1 msec, 50 Hz, 10 V) in the following experiments.

Influence of cerebral ischemia on the facilitatory modulation of the vagal baroreflex by the septum

Influence of the septal stimulation on phenylephrine-induced reflex bradycardia was assessed before 5-min global cerebral ischemia and during the reperfusion period of 60 to 90 min or at the corresponding time in the sham-operated animals. As shown in Fig. 3A, the facilitatory effect of

![Fig. 1. Example of the influence of the electrical stimulation of the septum on phenylephrine-induced reflex bradycardia in the anesthetized dog. BP: arterial blood pressure, MBP: mean arterial blood pressure, HR: heart rate.](image)

![Fig. 2. Amplitude- and frequency-response curves of the facilitatory effect of the septal stimulation on phenylephrine-induced reflex bradycardia. The doses of phenylephrine were 4.3±0.4 (left) and 5.8±1.0 (right) μg/kg, i.v., respectively. Ratio of the phenylephrine-induced increase in pulse interval (ΔPI) to that in mean blood pressure (ΔMBP). The doses of phenylephrine before and after sham ischemia were 5.0±1.2 and 8.5±0.8 μg/kg, i.v., respectively. The doses of phenylephrine before ischemia and during the reperfusion period were 5.0±0.7 and 10.2±2.2 μg/kg, i.v., respectively. Responses in the absence (□) or presence (□□) of the septal stimulation (1 msec, 50 Hz, 10 V) are indicated as the mean±S.E.M.](image)

![Fig. 3. Influence of 5-min global cerebral ischemia on the facilitation of the reflex bradycardia by the electrical stimulation of the septum. A: sham treatment (n=6), B: 5-min ischemia (n=6). Phenylephrine-induced reflex bradycardia is expressed as the ratio of increase in the pulse interval (ΔPI) to that in mean blood pressure (ΔMBP). The doses of phenylephrine before and after sham ischemia (in A) were 5.0±1.2 and 8.5±0.8 μg/kg, i.v., respectively. The doses of phenylephrine before ischemia and during the reperfusion period (in B) were 5.0±0.7 and 10.2±2.2 μg/kg, i.v., respectively. Responses in the absence (□) or presence (□□) of the septal stimulation (1 msec, 50 Hz, 10 V) are indicated as the mean±S.E.M. **P<0.01: significantly different from the value in the absence of the septal stimulation. *P<0.05, **P<0.01: significantly different from the corresponding value before ischemia.](image)
the septal stimulation on baroreflex was confirmed to be reproducible during the experimental period. On the other hand, as shown in Fig. 3B, the extent of reflex bradycardia during the reperfusion period was significantly smaller than that before ischemia. Furthermore, the septal stimulation failed to facilitate the reflex bradycardia in animals subjected to cerebral ischemia.

**DISCUSSION**

The present study demonstrates that 5-min global cerebral ischemia abolishes the facilitatory effect of intracisternal clonidine on the vagal reflex bradycardia. On the other hand, the hypotensive and bradycardic responses to clonidine were still observed, although attenuated, following ischemia. The latter result may be closely related to our previous observation that the sympathetic component of the baroreflex was resistant to the same ischemic insult as used in the present study (1). Thus, the residual responses to clonidine may be ascribed to the inhibition of the sympathetic activity (10–12).

Since clonidine was administered into the cisterna magna in the present study, the observed effects of clonidine may be mediated mainly by the structures in the medulla oblongata. In this context, dense distribution of parac佯romedullary binding sites has been observed in the dorsal motor complex including the nucleus tractus solitarius (NTS), the nucleus commissuralis (NC) and the dorsal motor nucleus of the vagus (DMV) in rats and humans (13). Furthermore, Gunn et al. (14) showed that the vagal preganglionic fibers in dogs arise from the DMV and the nucleus ambiguus in the medulla oblongata (13). Therefore, it can be speculated that ischemia might produce a certain functional impairment in the site of action of clonidine within the dorsal motor complex. Since NTS and NC form the first synapse in the baroreflex loop and contribute to both the sympathetic and vagal components (13, 15), the DMV would be the site mostly likely responsible for the attenuated vagal responses to clonidine following cerebral ischemia.

The present study, together with our recent observation that α2-adrenoceptor blocking agents prevent the post-ischemic dysfunction of the vagal baroreflex (2), suggests that excessive activation of α2-adrenoceptors during ischemia and the early reperfusion period may be deleterious for the function of the α2-adrenergic system within the medullary baroreflex pathway. In this regard, the increase in noradrenergic activity has been considered to be deleterious for ischemic brain injury in some studies (16, 17), while beneficial in others (18–22). Thus, the pathological consequences of the excessive activation of the noradrenergic system seem to depend on the experimental systems.

Although further studies are necessary to clarify the subcellular mechanisms which can explain the post-ischemic dysfunction of the α2-adrenergic system and the vagal baroreflex in our experimental model, one plausible mechanism is the desensitization or down-regulation of α2-adrenoceptors (23–26) by ischemia-induced increases in extracellular concentration or turnover of norepinephrine in the brain (27–29). In accord with this speculation, a decrease in the number of α2-adrenoceptor (idazoxan binding site) has been recently observed in rat temporal sensory cortex and amygdaloid at 60-min reperfusion following 15-min global cerebral ischemia (30). Additionally, DMV, which may be responsible for the attenuated response to clonidine in the present study as mentioned above, is innervated by the adrenergic nerves from the ventrolateral medulla (31, 32).

The present study also demonstrates that the post-ischemic attenuations of the vagal baroreflex and the responses to intracisternal clonidine are accompanied by the impairment of the response of the baroreflex system to the septal stimulation. The anatomical study in rats by Calaresu et al. (33) showed that the septum provides caudal projections mainly through the stria medullaris and the medial forebrain bundle. The precise anatomical pathways to or sites of connection with the primary baroreflex pathway within the medulla oblongata are still obscure, although Gebber and Klevans (34) suggested that the facilitatory interaction must occur at a site within the baroreflex pathway which is functionally close to the origins of the preganglionic cardiac vagal fibers.

In the previous study, we showed that the extent of the post-ischemic dysfunction of the baroreflex is correlated with the severity of ischemia in the dorsal medulla oblongata rather than the cerebral cortex (8). However, no data is available concerning the ischemic conditions in the regions between the cerebral cortex and the medulla oblongata. Therefore, the site of damage responsible for the impaired response to the septal stimulation is still obscure. Whatever the site of damage is, however, it is likely that the impairment of the facilitatory modulation from the forebrain structures may, at least in part, contribute to the post-ischemic dysfunction of the vagal baroreflex, considering the previous studies. For example, Klevans and Gebber (7) showed in cats that the electrical stimulation of the septum facilitates the vagal, but not sympathetic, component of reflex bradycardia, which was confirmed in the present study in dogs. Furthermore, bilateral destruction of the anterior hypothalamus, which is postulated to be another facilitatory center, attenuates the vagal reflex bradycardia without affecting the reflex inhibition of the sympathetic activity in rats (35).

In conclusion, the present study suggests that 5-min global cerebral ischemia may produce the dysfunction of
the neurons that are closely related to the baroreflex loop and receive the facilitatory modulation through $\alpha_2$-adrenoceptors and/or from the forebrain structures, leading to the selective dysfunction of the vagal baroreflex.

Acknowledgments
This study was partly supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (No. 02770110). The authors express their thanks to Mr. Masahiro Ashida, Mr. Shoichiro Sakamaki, Miss Makiko Yamada and Miss Nahoko Wakabayashi for their technical assistance.

REFERENCES

1 Kurihara, J., Sahara, T., Oda, N., Tomita, H. and Kato, H.: Selective dysfunction of the vagal baroreflex following cerebral ischemia: protection by ifenprodil and flunarizine. Eur. J. Pharmacol. 190, 23–30 (1990)

2 Tamaoki, S., Kurihara, J. and Kato, H.: Mechanism of the protective effect of ifenprodil against the dysfunction of baroreflex following cerebral ischemia. Japan. J. Pharmacol. 58, Supp. 1, 109P (1992)

3 Kurihara, J., Sahara, T., Tamaoki, S. and Kato, H.: MK-801 prevents the post-ischemic cerebral hypoperfusion, but not the dysfunction of the vagal baroreflex in dogs. Japan. J. Pharmacol. 59, 243–245 (1992)

4 Kobinger, W. and Walland, A.: Evidence for a central activation of a vagal cardiodepressor reflex by clonidine. Eur. J. Pharmacol. 19, 203–209 (1972)

5 Antonaccio, M.J., Robson, R.D. and Kerwin, L.: Evidence for increased vagal tone and enhancement of baroreceptor reflex activity after xyazine (2-2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazine) in anesthetized dogs. Eur. J. Pharmacol. 23, 311–315 (1973)

6 Laubie, M., Schmitt, H. and Drouillat, M.: Action of clonidine on the baroreceptor pathway and medullary sites mediating vagal bradycardia. Eur. J. Pharmacol. 38, 293–303 (1976)

7 Klevans, L.R. and Gebber, G.L.: Facilitatory forebrain influence on cardiac component of baroreceptor reflexes. Am. J. Physiol. 219, 1235–1241 (1970)

8 Kurihara, J., Sahara, T. and Kato, H.: Deterioration of baroreflex by transient global cerebral ischemia: Its correlation with the degree of ischemia or post-ischemic hypoperfusion in the medulla oblongata. Japan. J. Pharmacol. 51, 493–499 (1989)

9 Lim, R.K.S., Liu, C.-N. and Moffitt, R.L.: A Stereotaxic Atlas of the Dog's Brain. Charles C. Thomas Publisher, Springfield (Illinois) (1960)

10 Schmitt, H. and Schmitt, H.: Interactions between 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St155, CATAPRESAN®) and $\alpha$-adrenergic blocking drugs. Eur. J. Pharmacol. 9, 7–13 (1970)

11 Haemusler, G.: Activation of the central pathway of the baroreceptor reflex, a possible mechanism of the hypotensive action of clonidine. Naunyn Schmiedebergs Arch. Pharmacol. 278, 231–246 (1973)

12 Baum, T. and Shropshire, A.T.: Susceptibility of spontaneous sympathetic outflow and sympathetic reflexes to depression by clonidine. Eur. J. Pharmacol. 44, 121–129 (1977)

13 Unnerstall, J.R., Kopajtic, T.A. and Kuhar, M.J.: Distribution of $\alpha_2$ 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. 7, 69–101 (1984)

14 Guan, C.G., Sevelias, G., Puiggari, M.J. and Myers, F.K.: Vagal cardiomotor mechanisms in the hindbrain of the dog and cat. Am. J. Physiol. 214, 258–262 (1968)

15 Sper, K.M.: Central nervous integration of cardiovascular control. J. Exp. Biol. 100, 109–128 (1982)

16 Busto, R., Harik, S.I., Yoshida, S., Scheinberg, P. and Ginsberg, M.D.: Cerebral norepinephrine depletion enhances recovery after brain ischemia. Ann. Neurol. 18, 329–336 (1985)

17 Werner, C., Hoffman, W.E., Thomas, C., Miletich, D.J. and Albrecht, R.F.: Ganglionic blockade improves neurologic outcome from incomplete ischemia in rats: Partial reversal by exogenous catecholamines. Anesthesiology 73, 923–929 (1990)

18 Blomqvist, P., Lindvall, O. and Wieloch, T.: Lesions of the locus coeruleus system aggravate ischemic damage in the rat brain. Neurosci. Lett. 58, 353–358 (1985)

19 Koide, T., Wieloch, T.W. and Siesjö, B.K.: Circulating catecholamines modulate ischemic brain damage. J. Cereb. Blood Flow Metab. 6, 559–565 (1986)

20 Araki, T., Kogure, K. and Izumiya, K.: Prevention of ischemic neuronal damage by $\alpha_2$-adrenoceptor agonist (methoxamine). Acta Neuro. Scand. 80, 451–454 (1989)

21 Miyauuchi, Y., Wieloch, T. and Lindvall, O.: Noradrenaline metabolism in neocortex and hippocampus following transient forebrain ischemia in rats: relation to development of selective neuronal necrosis. J. Neurochem. 53, 408–415 (1989)

22 Hoffman, W.E., Cheng, M.A., Thomas, C., Baughman, V.L. and Albrecht, R.F.: Clonidine decreases plasma catecholamines and improves outcome from incomplete ischemia in the rat. Anesth. Analg. 73, 460–464 (1991)

23 Convents, A., De Backer, J.-P., André, C. and Vauquelin, G.: Desensitization of $\alpha_2$-adrenergic receptors in NG108 15 cells by (-)-adrenaline and phorbol 12-myristate 13-acetate. Biochem. J. 262, 245–251 (1989)

24 Jones, S.B., Leone, S.L. and Bylund, D.B.: Desensitization of the alpha-2 adrenergic receptor in HT29 and opossum kidney cell lines. J. Pharmacol. Exp. Ther. 254, 294–300 (1990)

25 Liggett, S.B., Ostrowski, J., Chesnut, L.C., Kurose, H., Raymond, J.R., Caron, M.G. and Lefkowitz, R.J.: Sites in the third intracellular loop of the $\alpha_2$, adrenergic receptor confer short term agonist-promoted desensitization. Evidence for a receptor kinase-mediated mechanism. J. Biol. Chem. 267, 4740–4746 (1992)

26 Eason, M.G. and Liggett, S.B.: Subtype-selective desensitization of $\alpha_2$-adrenergic receptors. Different mechanisms control short and long term agonist-promoted desensitization of $\alpha_2$C10, $\alpha_2$C4 and $\alpha_2$C2. J. Biol. Chem. 267, 25473–25479 (1992)

27 Globus, M.Y.-T., Busto, R., Dietrich, W.D., Martinez, E., Convens, A., De Backer, J.-P., Andre, C. and Vauquelin, G.: Subtype-selective desensitization of $\alpha_2$C10, $\alpha_2$C4 and $\alpha_2$C2. J. Biol. Chem. 267, 25473–25479 (1992)

28 Gustafson, I., Westerberg, E.J. and Wieloch, T.: Extracellular brain cortical levels of noradrenaline in ischemia: Effects of desipramine and postischemic administration of idazoxan. Exp. Brain Res. 86, 555–561 (1991)
29 Gustafson, I., Lidén, A. and Wieloch, T.: Brain cortical tissue levels of noradrenaline and its glycol metabolites: Effects of ischemia and postischemic administration of idazoxan. Exp. Brain Res. 90, 551–556 (1992)
30 Gustafson, I., Westerberg, E.J. and Wieloch, T.: Effects of ischemia on regional ligand binding to adrenoceptors in the rat brain. J. Neurol. Sci. 113, 165–176 (1992)
31 Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Supp. 367, 1–48 (1971)
32 Armstrong, D.M., Ross, C.A., Joh, T.H., Pickel, V.M. and Reis, D.J.: Distribution of dopamine-, noradrenaline- and adrenaline-containing cell bodies in the rat medulla oblongata demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. J. Comp. Neurol. 212, 173–187 (1982)
33 Calaresu, F.R., Ciriello, J. and Mogenson, G.J.: Identification of pathways mediating cardiovascular responses elicited by stimulation of the septum in the rat. J. Physiol. (Lond.) 260, 515–530 (1976)
34 Gebber, G.L. and Klevans, L.R.: Central nervous system modulation of cardiovascular reflexes. Fed. Proc. 31, 1245–1252 (1972)
35 Miyajima, E. and Buñag, R.D.: Anterior hypothalamic lesions impair reflex bradycardia selectively in rats. Am. J. Physiol. 248, H937–H944 (1985)