The Journal of Experimental Biology 207, 411-416
Published by The Company of Biologists 2004
doi:10.1242/jeb.00778

Stimulation of the α-adrenoceptor triggers the venom production cycle in the venom gland of *Bothrops jararaca*

Celine M. Kerchove[1], Sylvia M. Carneiro[2], Regina P. Markus[3] and Norma Yamanouye[1,*]

[1]*Laboratório de Farmacologia* and [2]*Laboratório de Biologia Celular, Instituto Butantan, Av. Vital Brazil, 1500, 05503-900, São Paulo, Brazil*, and [3]*Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, 05508-900 São Paulo, Brazil*

*Author for correspondence (e-mail: norma@butantan.gov.br)

Accepted 30 October 2003

Summary

The noradrenergic innervation of *Bothrops jararaca* venom gland is thought to be important in the production and secretion of venom. We investigated the characteristics of the α-adrenoceptor in the venom gland and its role in venom production. This receptor had relatively low sensitivity to noradrenaline (pD_2=4.77±0.09, *N*=7) and to phenylephrine (pD_2=3.77±0.06, *N*=11). The receptor became desensitized just after venom extraction (pD_2 to phenylephrine fell to 3.27±0.02, *N*=6) and the sensitivity remained low for at least 15 days, returning to normal 30 days after venom extraction, by which time the snake was ready for a new cycle of venom production. Incubation of secretory cells with noradrenaline (10^{-4} mol l^{-1} for 5 min) reduced α-adrenoceptor sensitivity to the level seen after venom extraction. Blockade of catecholamine production with reserpine abolished the enlargement of the rough endoplasmic reticulum and the activation of the Golgi apparatus that are normally seen after venom extraction, and the venom production was restored by a single subcutaneous (s.c.) injection of phenylephrine (100 mg kg^{-1}) immediately after venom extraction. Our data suggest that stimulation of the α-adrenoceptor during or shortly after biting is essential for the onset of the venom production cycle.

Key words: α-adrenoceptor, desensitization, venom production, venom gland, *Bothrops jararaca*, snake.

Introduction

Bothrops jararaca, a Brazilian solenoglyphous venomous snake, belongs to the subfamily Crotalinae, family Viperidae. The venom glands of viperid snakes are related to salivary glands (Kochva and Gans, 1970) with the peculiarity of having a central lumen where the venom produced accumulates (Kochva, 1960, 1987; Warshawsky et al., 1973; Mackessy, 1991). The viperid venom gland contains three distinct cell types: secretory cells, horizontal cells and mitochondria-rich cells. The secretory cells are the main component of the gland epithelium and comprise more than 80% of the total cell population (Kochva, 1978; Warshawsky et al., 1973).

After a bite or manual extraction of venom, the amount of venom inside the lumen decreases and the secretory epithelium undergoes morphological and biochemical changes. The epithelial cells change their shape from cuboid to columnar, the cisternae of the rough endoplasmic reticulum expand, and venom is synthesized. The maximal synthetic activity of the secretory cells and the highest mRNA concentration are observed 4–8 days after manual extraction. After that, the synthetic activity decreases and the venom is gradually accumulated in the gland lumen, while the epithelium returns to a quiescent stage (Ben-Shaul et al., 1971; Rotenberg et al., 1971; Oron and Bdollah, 1973; De Lucca et al., 1974; Kochva, 1978; Carneiro et al., 1991; Yamanouye et al., 1997). A complete venom production cycle lasts around 30–50 days.

The mechanisms that control the regulation of venom synthesis and secretion are not well understood. The noradrenenergic innervation of the venom gland appears essential for the venom production cycle because depletion of catecholamines by administration of reserpine blocks the cycle (Yamanouye et al., 1997). Both α- and β-adrenoceptors seem involved, as both the α-adrenoceptor agonist phenylephrine and the β-adrenoceptor agonist isoprenaline reverted many of the morphological changes in the venom gland seen after administration of reserpine (Yamanouye et al., 1997).

The aim of the current study was to investigate the role of the α-adrenoceptor during the venom production cycle in the *Bothrops jararaca* venom gland. Here, by functional studies using microphysiometry, we demonstrate that the α-adrenoceptor present in the snake venom gland has low sensitivity to noradrenaline and phenylephrine and undergoes a long-term desensitization after stimulation. We also show that stimulation of the α-adrenoceptor is essential for the onset of venom production.

Materials and methods

Animals and venom extraction

Adult *Bothrops jararaca* Weid 1824 of both sexes (*N*=44), weighing 100–400 g, were captured from the wild, classified by the Laboratory of Herpetology from Instituto Butantan, and treated and kept as described by Breno et al. (1990). These snakes had no access to food for 40 days to prevent loss of venom and to make sure that the cells were in quiescent state. It is important to point out that in the wild snakes can fast for 1–2 months but the fast can exceed 1 year (Secor and Nagy, 1994). For manual venom extraction (Belluomini, 1968), the snakes were anesthetized with sodium pentobarbital (20 mg kg^{-1}, s.c.). Animal care and procedures used were in accordance with the guidelines of the Animal Ethics Committee of Instituto Butantan.

Drugs

L-phenylephrine hydrochloride, (–)-arterenol bitartrate, (–)-propranolol hydrochloride, crystalline reserpine, hyaluronidase, antibiotics and antimycotic powder and 0.4% Trypan Blue solution were purchased from Sigma Chemical Co. (St Louis, MO, USA). Collagenase was purchased from Worthington Biochem. Co. (Lakewood, NJ, USA). Sodium pentobarbital was purchased from Cristália (São Paulo, Brasil). DMEM and foetal bovine serum were purchased from Gibco (Rockville, MD, USA). Other chemicals were of analytical or reagent grade, and were purchased from commercial suppliers.

Preparation of dispersed cells

Preparation of dispersed cells was based on the protocol described by Yamanouye et al. (2000) with some modifications. Briefly, snakes were anesthetized with sodium pentobarbital (30 mg kg^{-1}, s.c.), decapitated and the venom glands removed. The venom glands were freed from connective tissue and venom, and were cut into slices of 250 μm (McIlwain Tissue Chopper – Brinkmann). Cells were dispersed in an orbital shaker for 90 min at room temperature in Krebs–Hepes solution (composition in mmol l^{-1}: NaCl 120; KCl 4; $MgSO_4$ 1.2; KH_2PO_4 1.2; Hepes 15 and glucose 10; pH 7.4) containing collagenase (3 U mg^{-1} of wet tissue), hyaluronidase (3.5 U mg^{-1} wet tissue) and antibiotics and antimycotic (100 i.u. ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 0.25 μg ml^{-1} amphotericin B, respectively). After dispersion, the cells were washed with Dulbecco's Modified Eagle Medium (DMEM) containing sodium bicarbonate (40 mmol l^{-1}), antibiotics and antimycotic, filtered through a nylon mesh, then washed again and resuspended in DMEM containing 10% foetal bovine serum. All procedures were done under sterile conditions. Viable cells were counted in the presence of 0.4% Trypan Blue and 2×10^6 cells $well^{-1}$ were plated in a 24-well plate. The dispersed cells were cultured at 30°C in a humidified incubator (5% CO_2) for up to 2 days.

Measurement of extracellular acidification rate

Energy metabolism in living cells is tightly coupled to cellular ATP usage, so that any events that perturb cellular ATP levels, such receptor activation and initiation of signal transduction, will result in a change in acid excretion. Changes in the rate of cellular acid secretion have been used as a real-time indicator for changes in functional activity upon receptor stimulation in a wide variety of cells (Hafner, 2000), and have been used to characterize G protein-coupled receptor (Pihlavisto and Scheinin, 1999; MacLennan et al., 2000; Meloy et al., 2001).

Extracellular acidification rates were measured using a four-channel Cytosensor Microphysiometer system (Molecular Devices, Menlo Park, CA, USA). Dispersed cells were suspended in a 30% agarose solution and $0.5–1.0\times10^6$ cells were placed in a capsule cup. The capsule cups were loaded into the sensor chamber and the chambers were perfused at a flow of 100 μl min^{-1} with bicarbonate-free DMEM (containing 40 mmol l^{-1} NaCl and 0.57 mmol l^{-1} ascorbic acid) at 30°C. The cells were allowed to equilibrate for at least 45 min before exposure to agonists. The pump cycle time was 3 min, which included a 40 s pump-off period during which the acidification rate was measured. Drugs were diluted and perfused through a second fluid path to the sensor chamber. Cells were exposed to agonists for 1 min and a 30 min wash period was employed between successive agonist exposures. This stimulation protocol was found to induce reproducible responses in preliminary experiments. The rate of extracellular acidification was calculated by the Cytosoft Program (Molecular Devices Corporation, Sunnyvale, CA, USA). Changes in the rate of acidification were calculated as the difference between the maximum effect after agonist addition and the measurement taken immediately before agonist addition (basal acidification rate). Baseline acidification rates were normalized to 100% and changes due to agonist exposure were calculated as percent increases over normalized baseline.

Design of experiments

Response of venom gland cells to α-adrenoceptor agonists

Venom glands were removed from snakes from which no venom had been extracted for at least 40 days in order to have cells in a quiescent stage. Dispersed cells were placed in a Cytosensor system for the measurement of extracellular acidification rate. Dose–response curves were constructed for phenylephrine and noradrenaline. These experiments were done in the presence of 10^{-4} mol l^{-1} propranolol to block β-adrenoceptors present in the venom gland (Yamanouye et al., 1997, 2000). This dose of propranolol completely blocks the response of isoprenaline, a β-adrenoceptor agonist, in this preparation.

α-adrenoceptor sensitivity throughout the venom production cycle

We used snakes that had fasted for 40 days, and from which no venom was extracted during this period. After that, we extracted the venom and waited a variable period (<5 min, and 4, 7, 15 or 30 days) until the snake was killed and cells harvested from the venom gland. A control group was treated

similarly except that at the time of sacrifice no venom had been extracted for at least 40 days. Dispersed cells were placed in the Cytosensor system and dose–response curves to phenylephrine were constructed.

Effect of in vitro stimulation of α-adrenoceptors in the venom gland on the sensitivity of these receptors

Quiescent cells were harvested from snakes from which no venom had been extracted for at least 40 days. The dispersed cells were incubated for 5 min with a high concentration of noradrenaline (10^{-4} mol l^{-1}), kept at 30°C for 24 h, and placed in the Cytosensor system for the construction of dose–response curves to phenylephrine.

Stimulation of the α-adrenoceptor and cellular machinery involved in venom production

Snakes were injected with reserpine (20 mg kg^{-1} body mass, s.c.) to deplete endogenous catecholamine stores. Venom was extracted 24 h later, and venom extraction was immediately followed by an injection of phenylephrine (100 mg kg^{-1} body mass, s.c.) or vehicle. On the day of venom extraction and the subsequent 13 days, snakes continued to receive injections with reserpine (5 mg kg^{-1} day^{-1}, s.c.). 1 day after the last injection, the snakes were anesthetized and killed and their venom glands removed. The venom glands were prepared for electron microscopy as described by Carneiro et al. (1991). Ultrathin sections (70 nm) were analyzed using an LEO 906E transmission electron microscope.

Data analysis and statistics

Dose–response curves were fitted through a non-linear regression and pD_2 values ($-\log EC_{50}$) were calculated using the curve-fitting program Graph-Pad PRISM 3.0 (GraphPad Software, San Diego, CA, USA). The results are expressed as means ± S.E.M. of at least 3 independent experiments with 5–12 replicates each. Statistical significance ($P<0.05$) was assessed using Student's *t*-test to compare two values, or analysis of variance (ANOVA) followed by the Newman–Keuls test for multiple comparisons.

Results

Response of venom gland cells to α-adrenoceptor agonists

To stimulate α-adrenoceptors, we used noradrenaline or phenylephrine. To avoid stimulation of β-adrenoceptors, all experiments were done in the presence of propranolol, which is known to block increases in cAMP production in the venom gland induced by the β-agonist isoprenaline (Yamanouye et al., 2000). Phenylephrine is an α-adrenoceptor agonist in mammals, and reverses the effect of reserpine in the venom gland (Yamanouye et al., 1997), suggesting that it is an α-adrenoceptor agonist in snakes.

Noradrenaline and phenylephrine increased the rate of extracellular acidification in a dose-dependent manner in dispersed cells in the quiescent stage of the venom production cycle (Fig. 1). The pD_2 values were 4.77±0.09 (N=7) and 3.75±0.07 (N=11), respectively. The pD_2 values for phenylephrine in absence or presence of propranolol were not significantly different (data not shown).

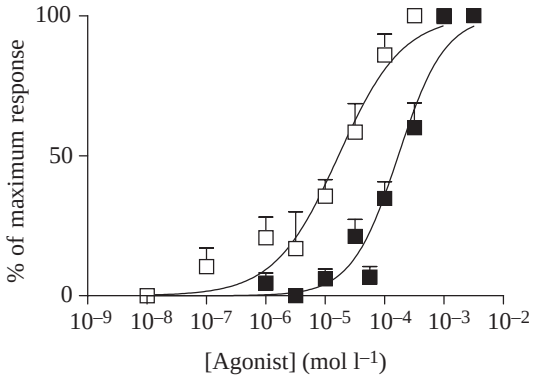

Fig. 1. Dose–response curves for noradrenaline (open squares, N=7) and phenylephrine (filled squares, N=11) in quiescent cells of the venom gland of *Bothrops jararaca*, as assessed by microphysiometry. Cultured cells were perfused with the agonists for 1 min, a complete dose–reponse curve was constructed in each well, and the interval between successive doses was 30 min. All experiments were done in the presence of the β-adrenoceptor blocker propranolol. Values are means ± S.E.M.

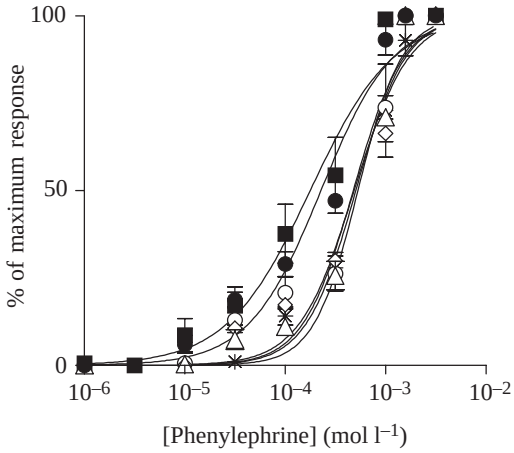

Fig. 2. Dose–response curves to phenylephrine in secretory cells in different stages of the venom production cycle: quiescent cells (filled squares, N=11), cells collected just after venom extraction (open triangles, N=6), cells collected 4 days (open circles, N=6), 7 days (open diamonds, N=5), 15 days (asterisks, N=6) and 30 days after venom extraction (filled circles, N=6). Note that venom extraction induced receptor downregulation that lasted for at least 15 days. Values are means ± S.E.M.

α-adrenoceptor sensitivity throughout the venom production cycle

In this experiment we measured the effect of the α-adrenoceptor agonist phenylephrine on venom gland cells that were harvested from the snake at various times after venom extraction. The pD_2 fell from 3.75±0.07 (N=11) in quiescent cells to 3.27±0.02 (N=6) immediately after venom extraction

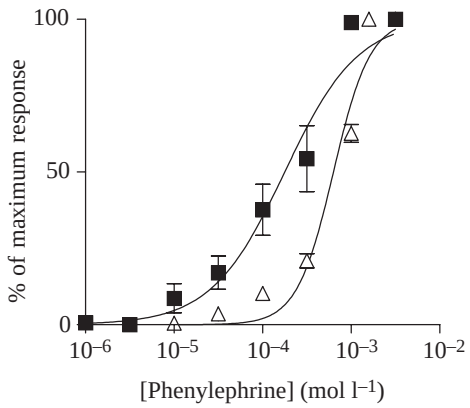

Fig. 3. *In vitro* desensitization of the α-adrenoceptor in cells of the *Bothrops jararaca* venom gland. Quiescent cells were incubated for 5 min with 10^{-4} mol l^{-1} noradrenaline and kept at 30°C for 24 h (triangles, *N*=6) before the construction of the dose–response curve to phenylephrine. Control quiescent cells (squares, *N*=11). Note that incubation with noradrenaline induced receptor downregulation. Values are means ± S.E.M.

(within 5 min, $P<0.05$, Fig. 2), and remained reduced for at least 15 days: day 4, 3.32±0.04 (*N*=6); day 7, 3.31±0.03 (*N*=5) and day 15, 3.29±0.04 (*N*=6), $P<0.05$). 30 days after venom extraction, pD_2 had returned to quiescent cells values (3.64±0.04, *N*=6).

Effect of in vitro stimulation of α-adrenoceptors in the venom gland on the sensitivity of these receptors

We hypothesized that the long-term desensitization might be due to stimulation of the α-adrenoceptor by noradrenaline released during venom extraction. To test if administration of noradrenaline desensitizes the α-adrenoceptors in the venom gland, quiescent cells were dispersed, incubated for 5 min with a high concentration of noradrenaline (10^{-4} mol l^{-1}), and kept at 30°C for the next 24 h. This treatment displaced the dose–response curve for phenylephrine to the right ($P<0.05$, Fig. 3). The pD_2 values in cells stimulated with noradrenaline (3.20±0.02, *N*=6) was close to the value seen in cells harvested soon after extraction of venom (3.27±0.02, *N*=6).

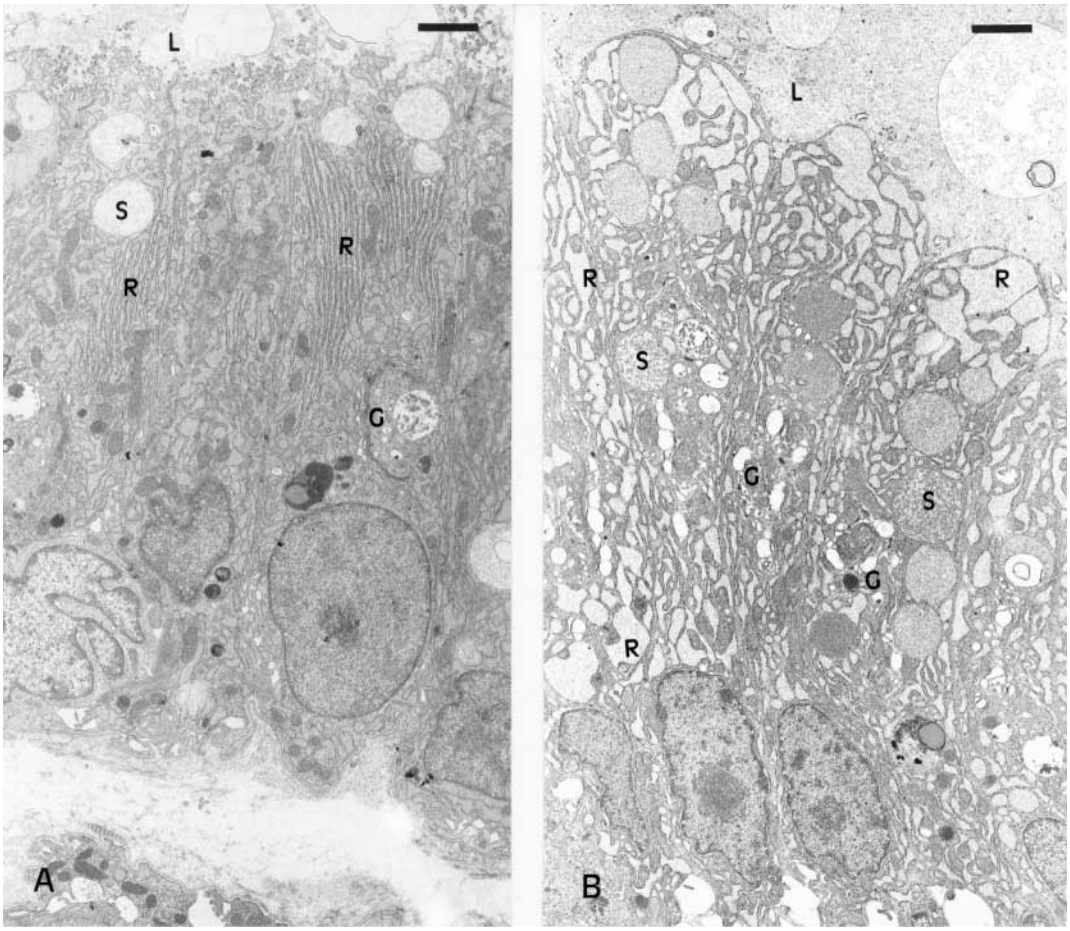

Fig. 4. Effect of a single dose of phenylephrine (100 mg kg^{-1} wet mass, s.c., *N*=3) on morphology of venom gland cells of reserpine-treated snakes. (A) Venom gland obtained from venom-extracted snakes after treatment with reserpine for 15 days. (B) Venom gland obtained from venom-extracted snakes after treatment with reserpine plus phenylephrine immediately after venom extraction. Note in A the narrow cisterna of the rough endoplasmatic reticulum indicative of lack of venom synthesis and a quiescent Golgi and in B the presence of wide rough endoplasmatic reticulum cisternae and several vesicles in the Golgi complex suggestive of venom production and secretion. G, Golgi apparatus; L, lumen; R, rough endoplasmic reticulum; S, secretory vesicle. Scale bars, 2 μm.

Stimulation of the α-adrenoceptor and cellular machinery involved in venom production

After venom extraction, the secretory cells increase in size and assume a columnar shape, the rough endoplasmic reticulum intracisternal spaces expand, many secretory vesicles appear near apical membrane and the Golgi apparatus become well developed (Yamanouye et al., 1997). In snakes that were treated with reserpine for 15 days, beginning the day before the extraction of venom, the cisternae of the rough endoplasmic reticulum were narrow and parallel and the Golgi apparatus appeared quiescent (Fig. 4A; Yamanouye et al., 1997). In a previous study we show that chronic treatment with phenylephrine (100 mg kg^{-1} body mass, s.c., 10 days after venom extraction) reverses the effect of reserpine on morphology of venom gland cells (Yamanouye et al., 1997). Considering that α-adrenoceptors undergo a long-term desensitization we hypothesized that their stimulation can trigger the venom production cycle. In this study, a single injection of phenylephrine (100 mg kg^{-1} body mass, s.c.) given immediately after the extraction of venom, reversed the effect of reserpine: the morphology of venom gland cells after this treatment (Fig. 4B) seemed similar to that of untreated cells. This corroborates our hypothesis about the importance of the α-adrenoceptors at the onset of the venom production cycle.

Discussion

In this report we show that the α-adrenoceptor present in the snake venom gland has a low sensitivity for noradrenaline and phenylephrine and undergoes a long-term desensitization. The sensitivity of the α-adrenoceptor in the venom gland of *Bothrops jararaca* is lower than the value we reported for the α-adrenoceptor in the aorta of this snake (Yamanouye et al., 1992; Yamanouye and Picarelli, 1992), suggesting that this snake expresses different subtypes of α-adrenoceptors. The low sensitivity found for the α-adrenoceptor of the venom gland is similar to those found in rat vas deferens circular muscle or spleen (Caricati-Neto et al., 1992; Buckner et al., 1996).

Although the sensitivity of the α-adrenoceptor in the venom gland is low, it may have an important role because the amount of noradrenaline in the venom gland is high (approximately 3 ng mg^{-1} wet tissue; Yamanouye et al., 1997), much higher than in the salivary glands, trachea and heart of the rat or salivary glands and brain of the mouse (De Avellar and Markus, 1990; Murai et al., 1995). We have previously shown that the β-adrenoceptor in the venom gland also has a low sensitivity to β-adrenoceptor ligands (Yamanouye et al., 2000).

Our study shows that the α-adrenoceptor became desensitized immediately after the extraction of venom. Desensitization also occurred when venom gland cells were incubated for 5 min with 10^{-4} mol l^{-1} noradrenaline, which suggests that the α-adrenoceptor desensitization after venom extraction is due to release of noradrenaline in the venom gland. Whether this rapid desensitization was due to uncoupling or to internalization of the receptor is not clear. We have previously shown that β-adrenoceptors in the venom gland become desensitized by extraction of venom (Yamanouye et al., 2000), suggesting that this desensitization is due to uncoupling. The α-adrenoceptor remained desensitized for at least 15 days. The long time necessary for recovery of the response suggests that a downregulation process occurred. However, sensitivity was restored 30 days after the extraction of venom. Since both the venom production cycle and α-adrenoceptor resensitization take around 30 days, it seems that the machinery necessary for initiating a new cycle is only ready when the gland is full of venom and the secretory cells have entered the quiescent stage.

We have previously shown that the effect of reserpine on morphology of the venom gland can be blocked with a chronic treatment of phenylephrine (100 mg kg^{-1} body mass day, s.c., for 10 days). The current finding that a single dose of phenylephrine of 100 mg kg^{-1} body mass, given immediately after venom extraction, is as effective as chronic treatment supports our idea that release of noradrenaline in the venom gland is especially pronounced immediately after the extraction of venom or biting. In previous work we have shown that the stimulation of β-adrenoceptor increases cAMP production in quiescent but not in activated cells, showing its relevance to starting the venom production cycle (Yamanouye et al., 2000). Therefore, taking all these data together, we conclude that noradrenergic innervation in the *Bothrops jararaca* venom gland is crucial to trigger the venom production and secretion machinery.

In conclusion, when the venom gland is full of venom, the secretory cells are in the quiescent state. Biting or manual extraction of venom triggers the production of new venom, which involves a change in the shape of the cells of the secretory epithelium from cuboid to columnar, expansion of the cisternae of the rough endoplasmic reticulum, and the production of secretory vesicles by the Golgi apparatus. To start this cycle, the presence of noradrenaline is essential: no venom is produced in the presence of reserpine. However, our data show that it is sufficient to have noradrenaline present only in the beginning of the venom production cycle, and that the noradrenergic innervation is not essential once the venom production is on the way. Our data also suggest that extraction of venom induces the release of noradrenaline, which indicates that noradrenaline is a causative rather than a permissive factor in the start of the venom production cycle. Knowledge of the dynamic of the venom gland will be helpful for understanding cellular mechanisms involved in venom production and secretion, and would aid the future production of venom in culture. Moreover, this peculiar exocrine gland is an attractive preparation for the investigation of cellular mechanisms of protein synthesis and secretion.

We thank Dr Guus H. M. Schoorlemmer for comments on this manuscript and Wanda R. Carrela da Silva and Débora Aparecida Moura for technical assistance. This work was supported by grants from FAPESP (Fundação de Amparo à

Pesquisa do Estado de São Paulo) to N.Y. (97/07990-0, 02/00422-8). N.Y. and R.P.M. are supported by researcher fellowships from CNPq (Conselho Nacional de Pesquisa) and C.M.K. is supported by a FAPESP graduate fellowship. C.M.K. is a graduate student at the Department of Histology and Embriology, University of São Paulo.

References

Belluomini, H. E. (1968). Extraction and quantities of venom obtained from some Brazilian snakes. In *Venomous Animals and Their Venoms* (ed. W. Bucherl, V. Buckley and V. Deulofeu), pp. 97-117. New York, Academic Press.

Ben-Shaul, Y., Lifshitz, S. H. and Kochva, E. (1971). Ultrastructural aspects of secretion in the venom glands of *Vipera palaestinae.* In *Toxins of Animal and Plant Origin* (ed. A. De Vries and E. Kochva), pp. 87-105. London, Gordon/Breach.

Breno, M. C., Yamanouye, N., Prezoto, B. C., Lazari, M. F. M., Toffoletto, O. and Picarelli, Z. P. (1990). Maintenance of the snake *Bothrops jararaca* (Wied, 1824) in captivity. *The Snake* **22**, 126-130.

Buckner, S. A., Oheim, K. W., Morse, P. A., Knepper, S. M. and Hancock, A. A. (1996). α_1-Adrenoceptor-induced contractility in rat aorta is mediated by the α_{1D} subtype. *Eur. J. Pharmacol.* **297**, 241-248.

Caricati-Neto A., Sette, M. and Jurkiewicz, A. (1992). Increased density of α-adrenoceptors in vas deferens of spontaneously hypertensive rats (SHR), indicated by functional and receptor binding studies. *Eur. J. Pharmacol*. **218**, 51-58.

Carneiro, S. M., Pinto, V. R., Jared, C., Lula, L. A. B. M., Faria, F. P. and Sesso, A. (1991). Morphometric studies on venom secretory cells from *Bothrops jararacussu* (jararacuçu) before and after venom extraction. *Toxicon* **29,** 569-580.

De Avellar, M. C. and Markus, R. P. (1990). Neuronal uptake of noradrenaline in the rat isolated trachea: effect of ageing. *Comp. Biochem. Physiol.* **96C**, 287-290.

De Lucca, F. L., Haddad, A., Kochva, E., Rothschild, A. M. and Valeri, V. (1974). Protein synthesis and morphological changes in the secretory epithelium of the venom gland of *Crotalus durissus terrificus* at different times after manual extraction of venom. *Toxicon* **12**, 361-369.

Hafner, F. (2000). Cytosensor® Microphysiometer: technology and recent applications. *Biosens. Bioelectron.* **15**, 149-158.

Kochva, E. (1960). A quantitative study of venom secretion by *Vipera palaestinae*. *Am. J. Trop. Med. Hyg.* **9**, 381-390.

Kochva, E. (1978). Oral glands of the Reptilia. In *Biology of the Reptilia*, vol. 8B (ed. C. Gans), pp. 43-161. London, Academic Press.

Kochva, E. (1987). The origin of snakes and evolution of the venom apparatus. *Toxicon* **25**, 65-106.

Kochva, E. and Gans, C. (1970). Salivary glands of snakes. *Clin. Toxicol.* **3**, 363-387.

Mackessy, S. P. (1991). Morphology and ultrastructure of the venom gland of the northern Pacific rattlesnake *Crotalus viridis oreganus*. *J. Morphol.* **208**, 109-128.

MacLennan, S. J., Reynen, P. H., Martin R. S., Eglen R. M. and Martin, G. R. (2000). Characterization of human recombinant alpha(2A)-adrenoceptors expressed in Chinese hamster lung cells using extracellular acidification rate changes. *Br. J. Pharmacol.* **129**, 1333-1338.

Meloy, T. D., Daniels, D. V., Hegde, S. S., Eglen, R. M. and Ford, A. P. D. W. (2001). Functional characterization of rat submaxillary gland muscarinic receptors using microphysiometry. *Br. J. Pharmacol.* **132**, 1606-1614.

Murai, S., Saito, H., Masuda, Y., Itsukaichi, O. and Itoh, T. (1995). Basal levels of noradrenaline, dopamine, 5-hydroxytryptamine, and acetylcholine in the submandibular, parotid, and sublingual glands of mice and rats. *Archs. Oral Biol*. **40**, 663-668.

Oron, U. and Bdolah, A. (1973). Regulation of protein synthesis in venom gland of viperid snakes. *J. Cell Biol.* **56**, 177-190.

Pihlavisto, M. and Scheinin, M. (1999). Functional assessment of recombinant human α_2-adrenoceptors subtypes with Cytosensor microphysiometry. *Eur. J. Pharmacol.* **385**, 247-253.

Rotenberg, D., Bamberger, E. S. and Kochva, E. (1971). Studies on ribonucleic acid synthesis in the venom glands of *Vipera palaestinae* (Ophidia, Reptilia). *Biochem. J.* **121**, 609-612.

Secor, S. M. and Nagy, K. (1994). Bioenergetic correlates of foraging mode for the snakes *Crotalus cerastes* and *Masticophis flagellum*. *Ecology* **75**, 1600-1614.

Warshawsky, H., Haddad, A.,Gonçalves, R. P., Valeri, V. and De Lucca, F. L. (1973). Fine structure of the venom gland epithelium of the South American rattlesnake and radioautographic studies of protein formation by secretory cells. *Am. J. Anat.* **138**, 79-120.

Yamanouye, N., Britto, L. R. G., Carneiro, S. M. and Markus, R. P. (1997). Control of venom production and secretion by sympathetic outflow in the snake *Bothrops jararaca*. *J. Exp. Biol.* **200**, 2547-2556.

Yamanouye, N., Carneiro, S. M., Scrivano, C. N. and Markus, R. P. (2000). Characterization of β-adrenoceptors responsible for venom production in the venom gland of the snake (*Bothrops jararaca*). *Life Sci.* **67**, 217-226.

Yamanouye, N. and Picarelli, Z. P. (1992). Characterization of postjunctional alpha-adrenoceptors in the isolated aorta of the snake *Bothrops jararaca*. *Comp. Biochem. Physiol.* **103C**, 615-618.

Yamanouye, N., Salomão, L. C. and Picarelli, Z. P. (1992). Effects of catecholamines on the isolated aorta of the snake *Bothrops jararaca*. *Comp. Biochem. Physiol.* **101C**, 197-201.