nous organelles, such as rough and smooth endoplasmic reticulum, and small mitochondria (fig. 1). Numerous foldings on the cell surface form an intricate pattern involving cell membranes of neighboring lymphocytes; nevertheless, no cell junctions were found. Some of these cells contained strikingly electron-dense granules, which appeared as dense bodies and/or lysosomes, or exhibited a round or polygonal electron-dense head and a long narrow tail consisting of material with a lower electron density and recalling a multilayer system of membranes (fig. 2).

Discussion. Numerous reports have been made of the presence of interdigitating cells in the central and peripheral lymphoid organs of mammals[1]. Their presence has been pointed out by Kendall and Frazier in some birds[2] and by Fonfria et al. in the spotless starling *Sturnus unicolor*[3]. As for reptiles and amphibians, presumptive pro-IDCs and mature IDCs have recently been found in the thymus and spleen of the turtle *Mauremys caspica*[4], and Plytycz has identified them in the spleen of the frog *Rana esculenta*[5]. No information exists concerning other ectothermic vertebrates.

In order to identify the cells present in the marginal zone of the splenic lymphoid follicles of *B. calamita* as possible IDCs, these morphological parameters have been taken into account; the low electron density of their cytoplasm, the scarcity of cytoplasmic membranous organelles and, most importantly, the numerous foldings on the surface of the membranes, which form a labyrinthine system of interdigitations. These features have all been included in the morphological characterization of mammalian[1] and avian IDCs[3]. This cell-type has been considered a member of the mononuclear-phagocyte system, concerned with the presentation of antigens to T-lymphocytes[7-10]. Remarkably, in the spleen of *B. calamita*, IDCs were more frequent after immunization with sheep erythrocytes[11].

It is difficult to evaluate, however, the presence in these cells of conspicuous cytoplasmic granules. Two types of granules have been described in mammalian IDCs: some are small, electron-dense and lysosomic, while the others are similar to the Birbeck granules of the Langerhans cells of the skin. In the IDCs reported in *B. calamita*, the former type occurs, together with another granular population whose morphology has never been described before. The tails of these granules may bear a certain resemblance to a primitive Birbeck granule or to so-called 'cored-tubules' also described in some Langerhans cells[12], or even to granules described in IDCs of the coecal tonsil of *Sturnus unicolor*[13]. Thorbecke et al.[14], on the other hand, have suggested that IDCs containing Birbeck granules present in mammalian lymph nodes represent mobile Langerhans cells or their descendents, which migrate to the paracortex of the lymph node after antigenic stimulation of the skin, principally in the T-dependent immunocellular reaction. IDCs are involved in the endocytosis of ferritin-antiferritin complexes in the mammalian thymus[15]. In contrast, the electron-dense heads differ totally from granules previously described in macrophages, monocytes, IDCs or Langerhans cells. Further histochemical and immunological characterization is therefore necessary in order for these preliminary results to be confirmed.

* To whom all correspondence should be addressed: Departamento de Morfología Microscópica, Facultad de Biología, Universidad de León, León (Spain).

1 Veldman, A.J.P., and Kaiserling, E., in: The reticuloendothelial system. A comprehensive treatise, vol. 1, p. 381. Eds I. Carr and W.T. Daems. Plenum Press, New York 1980.
2 Kendall, M.D., and Frazier, J.A., Cell Tissue Res. *199* (1979) 37.
3 Fonfría, J., Barrutia, M.G., Villena, A., and Zapata, A., Cell Tissue Res. *225* (1982) 687.
4 Leceta, J., Ph. D. thesis, Complutense University of Madrid, Spain (1984).
5 Plytycz, B., Personal communication.
6 Barrutia, M.G., Leceta, J., Fonfría, J., Garrido, E., and Zapata, A., Am. J. Anat. *167* (1983) 83.
7 Veerman, A.J.P., Cell Tissue Res. *140* (1974) 247.
8 Veerman, A.J.P., and van Ewijk, W., Cell Tissue Res. *156* (1975) 416.
9 Kamperdijk, E.W.J., Raamayers, E.M., de Leeuw, J.H.S., and Hoefsmit, E.M.C., Cell Tissue Res. *192* (1978) 1.
10 Groscurth, P., Path. Res. Pract. *169* (1980) 212.
11 Barrutia, M.G., Villena, A., Gomariz, R.P., Razquin, B., and Zapata, A., Cell Tissue Res., *239* (1985) 435.
12 Kobayashi, M., and Hoshino, T., J. Electron Microsc. *28* (1979) 285.
13 Fonfría, J., Villena, A., and Zapata, A., submitted for publication.
14 Thorbecke, C.J., Silberberg-Synakin, I., and Flotte, T.J., J. invest. Derm. *75* (1980) 32.
15 Schutte, R., Kamperdijk, E.W.A., and Hoefsmit, E.C.M., Int. Rep. Cell Biol. *7* (1983) 323.

0014-4754/85/111393-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1985

Some species differences in cardiovascular responses to intravenously injected leucine-enkephalin

S. Koyama*, N. Terada, Y. Shiojima and T. Takeuchi

Yamanashi Medical College, Department of Physiology, Tamaho, Nakakoma, Yamanashi 409-38 (Japan), 26 November 1984

Summary. Studies were conducted to determine the cardiovascular responses to leucine-enkephalin (L-enk) in three different species of animals; rabbit, dog and monkey. All animals were anesthetized with pentobarbital sodium after sedation with ketamine. Mean blood pressure (MBP) and heart rate (HR) were simultaneously monitored. The pressor and HR responses to bilateral carotid occlusion (BCO) were determined before injection of L-enk. Increased MBP and HR due to BCO in monkey were significantly greater than in the other two animal groups. Following i.v. injection of L-enk (5–30 μg/kg), a significant fall in MBP occurred in all groups in a dose-dependent manner; however, the time course of changes in MBP in rabbits was significantly shorter than that in the other animal groups. Significant decreases in HR after the injection of L-enk occurred in rabbits and dogs, whereas increases in HR occurred in monkeys. These results show that some cardiovascular responses to L-enk may be species dependent. These different cardiovascular responses to L-enk may be at least partly related to species differences in baroreceptor reflex sensitivity.
Key words. Leucine-enkephalin; blood pressure; heart rate; species differences.

The basic characteristics of cardiovascular responses to opioid peptides have been studied extensively in a number of species including the rat [2,4,11,14-16,18], the cat[5,6,9,14,19], and the dog[7,10,12,13]. Opioid peptides produce changes in systemic blood pressure and heart rate after either intravenous injection[9,12-14,18,19] or administration into the central nervous system[1,5,11,14,19]; however,

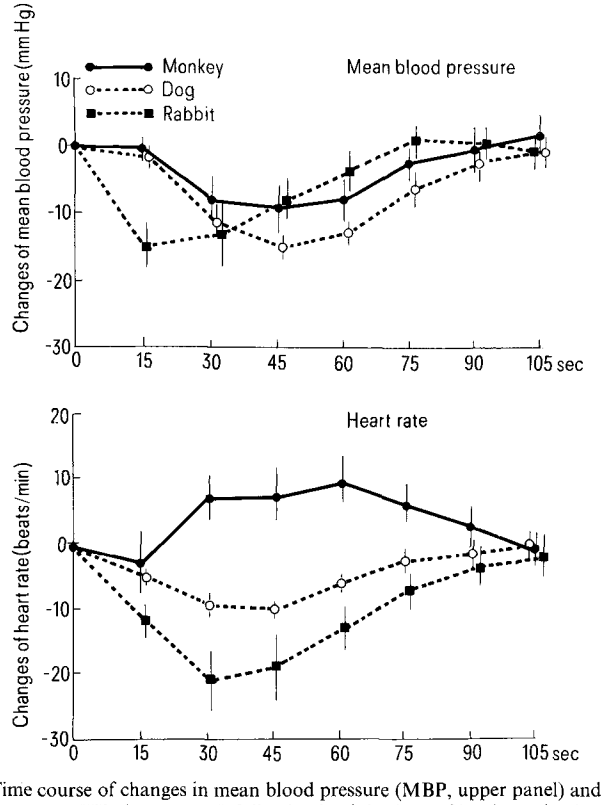
the nature of the hemodynamic responses to opioid peptides has been inconsistent. These inconsistencies in the reported responses to opioid peptides may also depend on species differences. However, there are few reports in the literature examining the cardiovascular effects of opioid peptides on different species of animals including the nonhuman primate. The present experiments were undertaken to determine whether the cardiovascular responses to such opioid peptides as enkephalins are dependent on the species of animal.

Methods. Experiments were performed on six rabbits of the New Zealand strain (1.8–2.2 kg), six adult mongrel dogs (8.2–15.4 kg) and six *Macaca fascicularis* monkeys (3.3–5.8 kg). All animals were anesthetized with i.v. injections of pentobarbital sodium (25 mg/kg) after a sedating dose of ketamine (5 mg/kg, i.m.). The trachea was intubated and respiration was maintained with a Harvard respirator. Body temperature was maintained throughout each experiment (rabbits, 39 ± 1 °C; dogs and monkeys, 36 ± 1 °C) by means of a heating lamp. A polyethylene tube was placed in the lower abdominal aorta through a femoral artery and connecting it to a pressure transducer for measurement of systemic blood pressure. A femoral vein was cannulated for administering drugs. Heart rate was determined from a continuously recorded limb lead electrocardiogram. Both external carotid arteries were exposed to determine the pressor and heart rate responses to bilateral carotid occlusion (BCO) during 30 s at 5 min before injection of enkephalin. Each animal was given a single injection of leucine-enkephalin (L-enk, 5–30 μg/kg; Protein Institute, Osaka, Japan). Mean blood pressure (MBP) and heart rate (HR) were monitored for 20 min following each injection of L-enk. Naloxone (1 mg/kg; Sankyo Co., LTD, Tokyo, Japan) was dissolved in physiological saline and injected over 60 s; 10 min later, injections of L-enk were repeated.

All values in this study are reported as the mean ± SE. All comparisons were made by analysis of variance and with paired or unpaired t-test. The difference between means was considered significant for $p < 0.05$.

Results. Baseline values of mean blood pressure (MBP) and heart rate (HR) in each animal group are shown in table 1. Changes in MBP and HR evoked by BCO are also shown in table 1. The MBP and HR responses to BCO in monkeys were significantly higher than in both dogs and rabbits. As shown in table 2, MBP and HR responses to L-enk occurred in proportion to dosage in the range from 5 to 30 μg/kg.

Following i.v. injections of L-enk at a dosage of 30 μg/kg, MBP in monkeys and dogs fell significantly so that 45 s after the injection changes in MBP were −9 ± 4 mm Hg and −15 ± 2 mm Hg respectively (fig.). Within 3 min MBP had returned to control levels. The time course of changes in MBP did not significantly differ between monkeys and dogs. MBP in rabbits also decreased significantly after the injection of L-enk, so that 15 s after the injection MBP reached a peak decrease of −14 ± 4 mm Hg. Changes in MBP 15 s after the injection in rabbits, however, was significantly different from that in monkeys and dogs (upper panel of the figure). I.v. injection of L-enk caused a rapid significant decreases in HR in rabbits and dogs. The maximum change in HR, in rabbits −21 ± 5 beats/min, occurred 30 s after the injection; in dogs the maximum change, −10 ± 2 beats/min,



Time course of changes in mean blood pressure (MBP, upper panel) and heart rate (HR, lower panel) following i.v. injections of leucine-enkephalin in monkeys, dogs and rabbits.

Table 1. Baseline values of mean blood pressure (MBP) and heart rate (HR), and changes in MBP and HR evoked by BCO, in each group

	Monkeys	Dogs	Rabbits
MBP (mm Hg)	102 ± 14	125 ± 5*	94 ± 3*
Δ MBP (mm Hg)	+45 ± 8	+31 ± 7*	+28 ± 9*
HR (beats/min)	108 ± 18	171 ± 5*	287 ± 13*
Δ HR (beats/min)	+37 ± 9	+25 ± 8*	+21 ± 27*

All values are mean ± SE. BCO = bilateral carotid occlusion. Δ MBP and Δ HR = changes in MBP and HR evoked by BCO, respectively. The asterisk (*) indicates a significant difference ($p < 0.05$) compared to monkeys.

Table 2. Changes in mean blood pressure (MBP) and heart rate (HR) due to the i.v. injection of leucine-enkephalin

	Monkeys	Dogs	Rabbits
Changes in MBP (mm Hg)			
5 μg	−2 ± 5	−4 ± 3	−3 ± 3
10	−5 ± 3	−8 ± 2	−7 ± 3
20	−6 ± 3	−10 ± 2	−11 ± 3
30	−9 ± 4	−15 ± 2	−14 ± 4
Changes in HR (beats/min)			
5 μg	−1 ± 4	−2 ± 4	−5 ± 3
10	5 ± 3	−4 ± 3	−10 ± 4
20	7 ± 3	−6 ± 4	−17 ± 3
30	10 ± 3	−10 ± 2	−21 ± 5

Mean ± SE.

Table 3. Changes in mean blood pressure (MBP) and heart rate (HR) due to leucine-enkephalin (30 μg/kg, i.v.) before and after pretreatment with naloxone

	Changes in MBP (mm Hg)		Changes in HR (beats/min)	
	Before	After	Before	After
Monkeys	− 9 ± 4	−1 ± 4*	10 ± 3	1 ± 3*
Dogs	−15 ± 2	−2 ± 5*	−10 ± 2	−1 ± 4*
Rabbits	−14 ± 4	−1 ± 5*	−21 ± 5	−3 ± 4*

Mean ± SE. The asterisk (*) indicates the statistical difference ($p < 0.05$) from values before the pretreatment with naloxone.

occurred. There was a significant difference between the peak decrease in HR in the two animal groups. In contrast, i.v. injection of L-enk in monkeys resulted in a significant rise in HR so that 60 s after the injection the change in HR was 10 ± 3 beats/min. There was a significant difference between the time course of changes in HR in monkeys and in the other two animal groups as illustrated in the lower panel of the figure. Naloxone prevented cardiovascular responses to L-enk in each animal group as shown in table 3.

Discussion. The results of this study showed that L-enk produced depressor effects in anesthetized rabbits, dogs and monkeys; however, the depressor response to L-enk in rabbits was significantly shorter in total time than that in monkeys and dogs. Additionally, in monkeys, L-enk caused a significant rise in HR in contrast to a significant fall in HR in rabbits and in dogs. These results suggest that the species of animal may be important in determining the cardiovascular action of such opioid peptides as L-enk. Inhibition of cardiovascular responses to L-enk by naloxone, as shown in the present study, indicates that the response is evoked by opioid receptors. Recently, it was reported that enkephalins and enkephalin analogs causes inhibition of the reflexly elicited decrease in heart rate evoked by pressor agents in conscious cats[14]. Therefore, the inconsistencies in the reported cardiovascular effects of the peptides may have resulted from different sensitivities of the arterial baroreceptor reflexes in different animal species used. The present study demonstrated that the pressor response to BCO was significantly greater in monkeys than in rabbits and dogs. It has been indicated also that there are species differences in the passive mechanical properties of carotid artery[3], and of carotid sinus baroreceptor sensitivity[17]. The results of the present study may explain why HR responses to L-enk in monkeys differ from those in rabbits and dogs.

Various enkephalins and enkephalin analogs caused a brief reduction in the systemic blood pressure and heart rate in anesthetized rats[1,11,18], cats[9,15,16] and dogs[7,13]. It has been reported that L-enk (35 μg/kg, i.v.) produces decreases in heart rate and systemic blood pressure in the pentobarbital anesthetized dog; however, it has been reported that in conscious animals these reductions of heart rate and systemic blood pressure are reversed and increase[12–14,19]. Therefore, the effects of anesthesia would appear to account for many of the inconsistencies in the reported action of the opioid peptides. It has since been demonstrated that administration of enkephalin analogs into the central nervous system caused increases in systemic blood pressure and heart rate in anesthetized rats[2,4,11]. These investigations suggest that the use of different routes for the administration of opioid peptides may also have been a cause of the inconsistensies in the reported cardiovascular effects of the opioid peptides, in addition to anesthetic agents and species differences.

Acknowledgments. The authors thank the staffs of the administration office of Yamanashi Medical College, chiefly Mr Hosaka, for administrative assistance in supplying the monkeys used in this study.

*Present address: Kagawa Medical School, Department of Physiology, Miki, Kita, Kagawa 761-07 (Japan).

1 Bolme, P., Fuxe, K., Agnati, L.F., Bradley, R.B., and Smythies, J., Eur. J. Pharmac. *48* (1978) 319.
2 Bellet, M., Elghozi, J.L., and Meyer, Ph., Archs Mal. Cœur *75* (1982) 37.
3 Cox, R.H., Am. J. Physiol. *234* (1978) H280.
4 Feuerstein, G., and Faden, A.I., Life sci. *31* (1982) 2197.
5 Floretz, J., and Mediavilla, A., Brain Res. *138* (1977) 585.
6 Hassen, A.H., Feuerstein, G.Z., and Faden, A.I., Life Sci. *31* (1982) 2193.
7 Laubie, M., Schmitt, H., Vincent, M., and Reynold, B., Eur. J. Pharmac. *46* (1977) 67.
8 Laubie, M., and Schmitt, H., Eur. J. Pharmac. *71* (1981) 401.
9 Moore III, R.H., and Dowling, D.A., Regulatory Peptides *1* (1980) 77.
10 Moss, I.R., and Scarpelli, E.M., J. appl. Physiol. *50* (1981) 1011.
11 Petty, M.A., and De Jong, W., Eur. J. Pharmac. *81* (1982) 449.
12 Sander, G.E., Giles, T.D., Kastin, A.J., Quiroz, A.C., Kaneish, A., and Coy, D.H., Peptides *2* (1981) 403.
13 Sander, G., Giles, T., Kastin, A., Kaneish, A., and Coy, D., Eur. J. Pharmac. *78* (1982) 467.
14 Schaz, K., Stock, G., Simon, W., Schlor, K-H., Unger, T., Rockhold, R., and Ganten, D., Hypertension *2* (1980) 395.
15 Sitsen, J.M.A., Van Ree, J.M., and De Jong, W., J. cardiovasc. Pharmac. *4* (1982) 883.
16 Summy-Long, J.Y., Rosella, L.H., and Keil, L.C., Brain Res. *221* (1981) 343.
17 Tomomatsu, T., Hufman, L., and Gilmore, J.P., Circulation Res. *52* (1983) 380.
18 Wei, E.T., Lee, A., and Chang, J.K., Life Sci. *26* (1980) 1517.
19 Yukimura, T., Stock, G., Stumpf, H., Unger, T., and Ganten, D., Hypertension *3* (1981) 528.

0014-4754/85/111394-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1985

Effect of nerve stimulation on rat skeletal muscle. A study of plasma membrane

A. Shah, F. Nagao, V. Sahgal and H. Singh

Neuromuscular Studies Unit, Rehabilitation Institute of Chicago, 345 E. Superior Street, Chicago (Illinois 60611, USA), 26 October 1984

Summary. The gastrocnemius muscle of the rat showed no morphological, histometric or plasma membrane changes, after sciatic nerve stimulation with a 5 mA current for 30 to 60 min, 10 mA for 30 min and 15 mA for 5 min. However, 10 mA for 60 and 200 min gave rise to mitochondrial and plasma membrane abnormalities. These changes were absent after a rest period. The results indicated that the sciatic nerve stimulation at 10 mA for 60 and 200 min caused reversible changes in the rat skeletal muscle mitochondria and plasma membrane.

Key words. Electrical stimulation; membrane; morphometry, muscle, ultrastructure.

There have been a number of reports on the chronic effects of direct electrical stimulation on denervated rat skeletal muscle[1–10]. However, there are no systematic studies of the immediate effects of electrical stimulation of nerve on normal skeletal muscle, especially with reference to plasma membrane changes. In the present investigation, we report on the immediate (acute) effects of electrical stimulation of sciatic nerve on the morphology, histochemistry, histometry and ultrastructure of rat gastrocnemius muscle. The state of the muscle plasma membrane was studied by lectin binding techniques.