fumigated at the predetermined sublethal and LD50 doses for 24 h in 2.5 l desiccators at 25–30 °C and 40–90% RH. In each experiment, there were 6 replicates each for control and sublethal dose, and 12 for LD50. At the end of the exposure period, mortality was found to be nil or negligible (< 1%) in control and sublethal treated batches. Survivors from each of 2 desiccators fumigated at LD50 were pooled, and from this pool about 300 mg were weighed and utilized for the enzyme assays.

Fumigated and control batches of insects (300 mg each) were separately homogenized at 0–5 °C in 3 ml of 0.1 M, 5.6 pH citrate buffer for trehalase, in 0.1 M NaF for phosphorylases and in 0.9% NaCl for AChE. The homogenate was centrifuged at 10,000 × g for 20 min. Trehalase assay was carried out according to Friedman[6], and the method of Sutherland and Wosilait[7] was followed for phosphorylases. Glucose was estimated by Nelson's method[8] and inorganic phosphate according to Taussky and Shorr[9]. Protein in the enzyme source was determined[10]. For AChE assay, the homogenate was directly used. About 10 ml of 0.015 M (final concentration) acetylcholine chloride and 1 ml of the insect homogenate were taken for the titrimetric assay of AChE[11].

Results and discussion. Both active and total phosphorylases were significantly inhibited in all the test insects (table). Trehalase was inhibited in *T. castaneum* adults exposed at sublethal and LD50 levels but in the larvae, the inhibition was noted only at LD50 dose. However, in Khapra larvae, one of the most tolerant stages for the majority of fumigants, trehalase activity was found to be least affected by acrylonitrile. The small effect on the AChE activity of the exposed insects is quite interesting. Acrylonitrile either alone or in a mixture with HCN increased AChE activity in nervous tissues of exposed rats[12]. The enhanced AChE activity in the brains of rats exposed to acrylonitrile has been related to the excitatory effect on the central nervous systems[4].

Trehalase activity, in control batches, was high in *T. castaneum* adults followed by its larvae and *T. granarium*. The reverse was true for phosphorylase activity. The activity of AChE in *T. castaneum* larvae was approximately twice that of adult stage; the Khapra larvae showed lowest activity. The low phosphorylases and AChE activities in control batches of *T. castaneum* adults may be attributed to inhibition by parabenzoquinones contained in their defensive secretions. These parabenzoquinones are known to be extremely reactive and they readily form addition compounds with proteins[13]. While preparing the homogenates with pestle and mortar at 0–5 °C, the penetrating odor of the defensive secretions of the beetles was observed. In fact, adults of *Tribolium* spp. are known to secrete parabenzoquinones under irritant conditions, i.e. on exposure to fumigants, in abnormal CO_2 or N_2 atmospheres and under cold stress[14,15]. Lord and Potter[16] suspected a material in extracts of *T. castaneum* adults (parabenzoquinones?) which inhibited hydrolysis of AChE.

1 Acknowledgments. We thank Dr R. Radhakrishnamurthy for his suggestions.
2 S. Friedman, Ann. Rev. Ent. *23*, 389 (1978).
3 W. Chefurka, in: The Physiology of Insecta. Academic Press, New York 1964.
4 G.M. Klimwa, Narusheniya Metab., Tr. Nauchn. Konf. Med. Inst. Zapadn. Sib. *46* (1974).
5 L.V. Zotova, Biol. Akt. Veshchestva (Mikroelem., Vitam. Drugie) Rastenievod., Zhivotnovod. Med. *98* (1975).
6 S. Friedman, Archs Biochem. Biophys. *87*, 252 (1960).
7 E.W. Sutherland and W.D. Wosilait, J. biol. Chem. *218*, 459 (1956).
8 N. Nelson, J. biol. Chem. *153*, 375 (1944).
9 H.H. Taussky and E. Shorr, J. biol. Chem. *153*, 675 (1953).
10 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
11 L.E. Chadwick, J.B. Lovell and V.E. Egner, Biol. Bull. *104*, 323 (1953).
12 N.E. Mikhailov, Biol. Akt. Veshchetva (Mikroelem., Vitam. Drugie) Rastenievod., Zhivotnovod, Med. *137* (1975).
13 R.K. Ladisch and B. McQue, Science *118*, 324 (1953).
14 L.M. Roth and R.B. Howland, Ann. ent. Soc. Am. *34*, 151 (1941).
15 D.G. Irwin, L.W. Smith and J.J. Pratt, J. stored Prod. Res. *8*, 213 (1972).
16 K.A. Lord and C. Potter, Nature *172*, 679 (1953).

Enkephalins induce asymmetrical effects on posture in the rat

E.I. Chazov, G.Ya. Bakalkin, K.N. Yarigin, E.D. Trushina, M.I. Titov and V.N. Smirnov[1]

USSR Research Centre of Cardiology, Academy of Medical Sciences, Petroverigsky Lane 10, Moscow 101837 (USSR), 29 October 1980

Summary. Enkephalins when applied subarachnoidally induce hind limb postural asymmetry in rats with transected spinal cord. The effect is antagonized by naloxone. Methionine-enkephalin induces predominantly left limb flexions while leucine-enkephalin causes mostly right limb flexions. The data suggest an asymmetry existing in the enkephalinergic system in the rat spinal cord.

Opioid peptides, endorphins and enkephalins have been found recently in brain and pituitary[2]. These substances are known to affect pain perception[3], behaviour[4] and some endocrine functions[5,6], and may also play a role in the control of muscular tone[7,8]. In this paper a new property of enkephalins is described; their ability to induce postural asymmetry of the hind limbs in rats with a transected spinal cord. We further report that the side of the asymmetry is dependent on the type of enkephalin used: methionine-enkephalin (Met-enkephalin) predominantly induces flexion of the left limb whereas leucine-enkephalin (Leu-enkephalin) induces flexion of the right limb. It is suggested that this influence may be due to asymmetric responsiveness of the spinal cord to enkephalins.

Methods. Enkephalins were injected into the subarachnoid space of the caudal portion of the spinal cord of 150–180 g male albino rats after laminectomy at the T5–T9 level and cord transection. Operations were carried out over the time interval from 10.00 h to 14.00 h under ether anaesthesia. The spinal cord was doubly ligated at T5–T9 and 20µl of saline (control) or enkephalin solution in saline (experimental) were administered subarachnoidally, 5 mm

caudally to the lower ligature. The spinal cord was transected between the ligatures and postural asymmetry was measured 0.5, 1, 4, 24 and 48 h after cord section. The resulting hind-limb postural asymmetry was manifested as flexion of the right or left hind leg in the hip and knee joints. The asymmetry was quantitated by measuring the length of the line connecting the projection of the position of the big toes of both hind limbs onto the longitudinal axis of the animal[9,10]. The onset of postural asymmetry, its magnitude in mm and the side of flexed hind limb were registered. Since intact rats occasionally (less than 10–20% of animals) showed postural asymmetries of 1–2 mm, only asymmetries exceeding 3 mm were registered in the experiments.

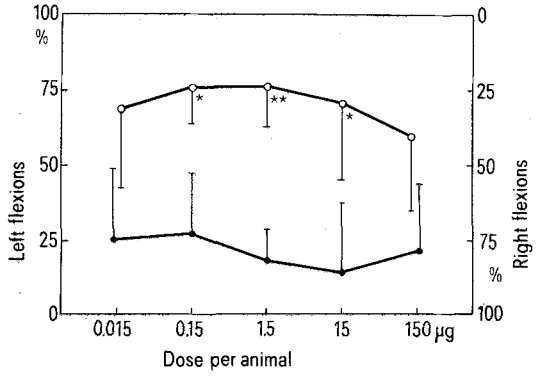
Naloxone was injected s.c. either 5 min before administration of the peptide (postural asymmetries were measured 24 h later) or 24 h thereafter (asymmetry was measured 60 min later). In these cases the following doses were used: 1.5 µg Met-enkephalin or Leu-enkephalin per animal (control); 1.5 µg Met-enkephalin and 825 µg naloxone per animal or 1.5 µg Leu-enkephalin and 330 µg naloxone per animal (experimental). Naloxone (825 µg per animal) injected s.c. into spinal rats prior or after subarachnoidal administration of saline did not induce hind-limb postural asymmetry.

The χ^2-test was applied to evaluate the statistical significance of the difference in the percentage of animals showing postural asymmetry in control and experimental groups.

Results and discussion. As shown in table 1, administration of either Met-enkephalin or Leu-enkephalin leads to the development of postural asymmetry. The percentage of animals exhibiting postural asymmetry depends upon the dose of the peptide used and is maximal (50–80%) after injection of 150 ng–150 µg of the peptide. The percentage of animals with postural asymmetry in the experimental groups given enkephalins is significantly higher (2–5-fold) than in the control animals given saline instead. No significant differences are observed between Met-enkephalin and Leu-enkephalin in the percentage of animals developing asymmetry. The percentage of animals with postural asymmetry reaches a maximum by 24 h after drug injection (data for this time interval are presented). None or few postural asymmetries develop during the 1st h following peptide administration. It seems likely that enkephalins, being themselves unstable, induce slow neurochemical processes causing delayed formation of an asymmetry of posture.

The opiate antagonist naloxone was used in order to examine whether opiate receptors are involved in the enkephalin-induced effects. As table 2 shows, the injection of naloxone 24 h after the administration of either enkephalin reduces the percentage of animals exhibiting postural asymmetry. Further, naloxone injected prior to Met-enkephalin decreases the fraction of animals developing postural asymmetry as compared to the control group that received Met-enkephalin alone (the effect of naloxone prior to Leu-enkephalin administration was not examined). Thus, it is likely that opiate receptors are involved in the development of asymmetry.

When the side of the flexed hind limb is taken into consideration, a new aspect of the enkephalin effects is



The percentage of animals with left or right hind-limb flexion induced by Met-enkephalin (○) and Leu-enkephalin (●). For experimental details and the number of animals exhibiting postural asymmetry see table 1. The χ^2 test and tables of confidence limits for probability were used for the statistical evaluation of the results. Bars show 95% confidence intervals. The statistical significance of differences between left flexion/right flexion ratios for equal doses of Met-enkephalin and Leu-enkephalin was evaluated. The results are shown in the figure: $^*p < 0.01$ and $^{**}p < 0.001$. Left to right flexion ratio induced by enkephalins was also compared with the distribution 50% left to 50% right flexions. These differences were also statistically significant ($p < 0.001$ for Met-enkephalin and $p < 0.0001$ for Leu-enkephalin). In this case the data for each peptide were pooled independent of the dose used, since no statistical difference was found between the left to right flexion ratio at various doses for either peptide.

Table 1. Induction of postural asymmetry by enkephalins

Drug	Dose per animal	Number of animals in the experiment	Percent of animals exhibiting postural asymmetry at 24 h
Saline		102	15
Met-enkephalin	1.5 pg	16	25
	1.5 ng	15	40
	15 ng	15	40
	150 ng	67	63**
	1.5 µg	55	62**
	15 µg	30	50**
	150 µg	23	83**
Leu-enkephalin	1.5 pg	15	33
	1.5 ng	10	60*
	15 ng	25	64**
	150 ng	33	54**
	1.5 µg	70	57**
	15 µg	25	52**
	150 µg	29	59**

The percentage of animals showing postural asymmetry reached a maximum by 24 h (data for this time interval are presented in the table). Postural asymmetries were measured under light ether or nembutal (40 mg/kg of b.wt) anaesthesia. Similar results were obtained with both anaesthetics. * $p < 0.01$; ** $p < 0.001$.

Table 2. Effect of naloxone on the induction of postural asymmetry by Met- or Leu-enkephalin

Drug	Number of animals in the experiment	Percent of animals exhibiting postural asymmetry
Saline	102	15[a]
Met-enkephalin	55	62[a]
Naloxone prior to Met-enkephalin	11	27*
Naloxone after Met-enkephalin	12	25*
Leu-enkephalin	70	57[a]
Naloxone after Leu-enkephalin	15	27*

* $p < 0.05$; [a] data from table 1.

disclosed (fig.). Over a broad dose range from 15 ng to 150 µg Met-enkephalin induces predominantly flexion of the left limb, whereas Leu-enkephalin causes mainly the right limb to flex. The difference in the left/right flexion ratio after administration of Met- and Leu-enkephalin is statistically significant. Moreover, for both enkephalins these values differ significantly from the theoretically random distribution of 50% left flexions to 50% right flexions. These facts suggest that the systems regulating the activity of spinal effector neurons located symmetrically to the sagittal plane may differ in their sensitivity towards Met- and Leu-enkephalin.

Opiate receptors have been found at all levels of the spinal cord in the posterior, lateral and anterior horns and in the intermediate zone of grey matter[11,12]. These receptors may be involved in the regulation of spinal motor reflexes through modulation of the activity of sensory, motor and internuncial neurons. This suggestion is supported by observations that morphine and other opiates affect electrical activity of spinal motoneurons and interneurons[7,13]and force of flexor reflex[14] in intact and spinal animals.

The appearance of hind-limb asymmetry induced by enkephalins in these experiments may indicate an intrinsic asymmetry either in the density or other properties of opiate receptors or in postreceptor elements involved in the enkephalin response. However, since the side on which flexion was induced depended on whether Met-enkephalin or Leu-enkephalin was administered the simplest interpretation is 1. that these receptors are relatively more specific for either of the opiates; and 2. that these receptors are asymmetrically distributed.

Asymmetric reaction and hemispheral asymmetry in the response of the nigro-striatal system to dopaminergic agonists have also been described by Glick et al.[15] These workers found that the left and right striata differ by some 10–15% in dopamine content. Amphetamine administration increased this difference up to 25% and induced rotation of the animal in the direction corresponding to the brain hemisphere with the higher dopamine content[16]. Pentamethylenetetrazol increased the amplitude of wave-spike discharges on the same side of the brain[17]. Our data suggest that in addition to the asymmetry of the dopaminergic system in the brain an asymmetry of the enkephalinergic system in the spinal cord exists.

1 Acknowledgments. The authors are grateful to Prof. J. Baxter for critical review of the manuscript and stimulating discussion.

2 H.W. Kosterlitz, and J. Hughes, Adv. Biochem. Psychopharmac. *18*, 31 (1978).
3 L. Terenius, Adv. Biochem. Psychopharmac. *18*, 321 (1978).
4 R. Guillemin, N. Ling, R. Burgus, F. Bloom and D. Segal, Psychoneuroendocrinology *2*, 59 (1977).
5 J.F. Bruni, D. Van Vugt, S. Marshall and J. Meites, Life Sci. *21*, 461 (1977).
6 H. Akil, S.J. Watson, P.A. Berger and J.D. Barchas, Adv. Biochem. Psychopharmac. *18*, 125 (1978).
7 U. Seeber, K. Kuschinsky and K.-H. Sontag, Naunyn-Schmiedeberg's Arch. Pharmak. *301*, 181 (1978).
8 F.E. Bloom, J. Rossier, E.L.F. Battenberg, A. Bayon, E. French, S.J. Henriksen, G.R. Siggins, D. Segal, R. Browne, N. Ling and R. Guillemin, Adv. Biochem. Psychopharmac. *18*, 89 (1978).
9 T.J. Chamberlain, P. Halick and R.W. Gerard, J. Neurophysiol. *26*, 662 (1963).
10 C. Guirgea and F. Mouravieff-Lesuisse, Archs int. Pharmacodyn. *191*, 279 (1971).
11 R. Simantov, M.J. Kuhar, G.R. Uhl and S.H. Snyder, Proc. natl Acad. Sci. USA *74*, 2167 (1977).
12 O. Johansson, T. Hökfelt, R.P. Elde, M. Schultzberg and L. Terenius, Adv. Biochem. Psychopharmac. *18*, 51 (1978).
13 D. Le Bars, G. Guilbaud, I. Jurna and J.M. Besson, Brain Res. *115*, 518 (1976).
14 W.R. Martin, C.G. Eades, J.A. Thompson, R.E. Huppler and P.E. Gilbert, J. Pharmac. exp. Ther. *197*, 517 (1976).
15 S.D. Glick, T.P. Jerussi, D.H. Waters and J.P. Green, Biochem. Pharmac. *23*, 3223 (1974).
16 S.D. Glick, T.P. Jerussi and L.N. Fleicher, Life Sci., *18*, 889 (1976).
17 M. Myslobodsky and J. Rosen, Epilepsia *20*, 377 (1979).

Effect of isoprenaline on dopamine receptors in the rabbit isolated renal artery

M.J. Kelly

School of Health and Applied Sciences, Leeds Polytechnic, Calverley Street, Leeds LS1 3HE (England), 12 December 1980

Summary. The relaxant effects of isoprenaline on rabbit isolated renal artery and aorta were compared. The results suggest that although isoprenaline acts on β-adrenoceptors in the aorta it stimulates dopamine receptors in the renal artery.

A number of in vivo studies have shown dopamine to be capable of dilating blood vessels in the renal[1-3], mesenteric[4], coronary[5], hindlimb[6] and paw pad[7] vasculatures. More recently, work with mesenteric, renal[8], coronary[9], cerebral[10] and splenic[11] isolated arterial strips, confirming the in vivo observations, has suggested the presence of a dopamine receptor, mediating a type of vasodilation which is unaffected by the presence of β-adrenoceptor antagonists[12].

Earlier studies had indicated that the β-adrenoceptor agonist isoprenaline acted to dilate renal blood vessels by stimulating β-adrenoceptors, since the response was blocked by β-adrenoceptor antagonists[1,2]. However, Bell and Mya[13] have questioned these observations and their in vivo study of the canine renal vasculature has led to the suggestion that isoprenaline induces renal dilatation, at least in part, by interacting with dopamine receptors. The present study seeks to clarify this observation by comparing isoprenaline's relaxant effects on the rabbit isolated renal artery with those on the aorta, where its β-adrenoceptor stimulating properties are well known[14,15].

Methods. Rabbit thoracic aorta and left renal artery were removed immediately after death and placed in Krebs-Henseleit solution, bubbled with a 95% oxygen/5% carbon dioxide gas mixture. The tissues were cut into spiral strips[16] and mounted vertically in 20 ml tissue baths containing oxygenated Krebs-Henseleit solution, maintained at 37 °C. Tissues were stretched under 1–2 g of resting tension for 1 h and then incubated with the α-adrenoceptor antagonist phenoxybenzamine (10 µM) for a further hour before drug-induced tension changes were recorded isometrically. Prostaglandin $F_{2\alpha}$ (3 µM) was used to induce muscle tone and relaxant drugs were administered cumulatively after the