Short Communication

Impaired Passive Avoidance Acquisition in Wistar Rats after Restraint/Cold Stress and/or Stresscopin Administration

V. Klenerová, O. Kaminský, P. Šída, Z. Hliňák, I. Krejčí and S. Hynie

Department of Pharmacology, First Medical Faculty, Charles University, Prague, Czech Republic

Abstract. Stresscopin (SCP) and related peptides are new members of the corticotropin-releasing factor (CRF) peptide family that are selective ligands for CRF type 2 receptor; these ligands are essential for maintaining homeostasis after stress. SCP (i.p. injections) was tested on the passive avoidance learning task in stressed Wistar rats; it impaired the formation of memory trace. The retention performance deficit induced by SCP was comparable with the deficit induced by the stressor of restraint/cold. More profound impairment of avoidance response occurred following combined application of SCP and stressor. More specific actions of SCP can be expected from its studies with targeted intracerebral applications.

Key words: Learning — Passive avoidance — Rats — Stresscopin — Stress

Impairment of learning and memory processes has been demonstrated by many studies using different stressors. It has been well established that hormones and neuromodulatory factors released by stress-induced activation of hypothalamo-pituitary-adrenocortical (HPA) axis influence neurobiological mechanisms underlying learning and memory formation (Herman and Cullinan 1997; Lupien and McEwen 1997; McGaugh and Roozendaal 2002). In passive avoidance task the performance of rats has been shown to depend on optimal levels of adrenocorticotropic hormone (ACTH), corticosteroids and adrenomedullar catecholamines; vasopressin and oxytocin influence passive avoidance learning as well. Relatively recently it has been demonstrated that also the corticotropin-releasing factor (CRF), which stimulates the release of ACTH, plays a crucial role in stress responses (Aguilera 1998; Coste et al. 2001; Smagin et al. 2001). The function of CRF and another releasing hormone – thyrotropin-releasing hormone (TRH) – can be studied by estimation of their levels and mRNA (Kiss and Jezova 2001; Petkova-Kirova et al. 2001) as well as by application of their analogs and measuring their physiological responses.

Correspondence to: Doc. MUDr. Věra Klenerová, DrSc., Charles University in Prague, First Medical Faculty, Department of Pharmacology, Albertov 4, 128 00 Prague 2, Czech Republic. E-mail: Vera.Klenerova@LF1.cuni.cz

There is evidence suggesting that CRF peptides play biologically diverse roles in generating the stress responses. These responses are caused by selective activation of CRF type 1 receptor ($CRFR_1$) and CRF type 2 receptor ($CRFR_2$) that are differentially expressed in various peripheral tissues and brain regions (Chan et al. 2000; Shi et al. 2000; Higelin et al. 2001; Reul and Holsboer 2002).

Stresscopin (SCP) and stresscopin-related peptide (SRP) are selective ligands for $CRFR_2$ (Hsu and Hsueh 2001). It has been demonstrated that i.p. injections of both hormones suppressed heat-induced edema formation in anesthetized rats (Hsu and Hsuesh 2001). Further, these substances decreased food intake and had an inhibitory effect on gastric emptying activity. The authors concluded that these two hormones might represent endogenous ligands for maintaining homeostasis after stress. It is difficult to guess which are the direct peripheral effects and how many of the central actions participate on these effects after systemic application of SCP.

Molecular characterization of $CRFR_1$ and $CRFR_2$, and preparation of novel ligands have revealed a far-reaching physiological importance for the family of CRF peptides (Dautzenberg and Hauger 2002). At present, the most studied CRF peptides are urocortin, stresscopin-related peptide/urocortin II and stresscopin/urocortin III (Skelton et al. 2000; Li et al. 2002). The last two peptides are exclusive $CRFR_2$ ligands. Since $CRFR_2$ agonists might represent endogenous ligands for maintaining homeostasis after stress, it is tempting to test these ligands in stress situations in order to evaluate the influence of the stress effects on behavioral model of cognitive processes. Therefore, we decided to estimate the effect of $CRFR_2$ agonist on stress induced memory impairment of rats using the passive avoidance paradigm. We used restraint and cold stress in the passive avoidance situation under experimental conditions that were repeatedly used in the previous studies (Klenerova et al. 2002). We administered human SCP that was recently studied in various pharmacological tests (Hsu and Hsueh 2001).

Adult mail Wistar (WI) rats (Biotest Ltd., Konarovice, Czech Republic), weighing 240 g, were maintained on a 12 hour light : 12 hour dark cycle, and had free access to a standard pellet food and water. Treatment of animals was in accordance with the Declaration of Helsinki Guiding Principles on Care and Use of Animals (DHEW Publication, NHI 80-23).

Rats were exposed to restraint (immobilization) combined with water immersion (IMO+C) in the water bath (22 ℃) for 60 min and then left undisturbed for 120 min (IMO+C60/120) in the home cage (Klenerová et al. 2002).

Human SCP (Hsu and Hsueh 2001) with the structure H-Thr-Lys-Phe-Thr-Leu-Ser-Leu-Asp-Val-Pro-Thr-Asn-Ile-Met-Asn-Leu-Leu-Phe-Asn-Ile-Ala-Lys-Ala-Lys-Asn-Leu-Arg-Ala-Gln-Ala-Ala-Ala-Asn-Ala-His-Leu-Met-Ala-Gln-Ile-NH_2 and molar mass 4367.2 was prepared in PolyPeptide Laboratories, Prague, Czech Republic. It was injected i.p. to rats in a dose of 100 nmol/kg (Hsu and Hsueh 2001) 60 min before the application of an aversive stimulus. A shuttle-cage (Coulbourn Instruments Inc., PA, USA) consists of two communicating compartments of equal size that are separated by a sliding door. The starting compartment was illumi-

nated and the shock compartment was dark. A stainless steel bar floor was used for delivery of scrambled constant current. WinLinc software was used for designing passive avoidance testing and to process experimental data.

The experiment started with two pre-training trials performed on two consecutive days. Each time rat was placed in the illuminated starting compartment for 50 s. After this interval the sliding door was raised and the latency to enter the dark compartment was recorded. During the single training trial (Day 3) the rats were placed in the illuminated compartment of the apparatus as in the previous sessions and the latency to enter the shock (dark) compartment was recorded. The door was then closed and a footshock (0.3 mA, 3 s) as aversive stimulus (AS) was delivered to the rat, which was removed from the dark compartment 1 min later.

Retention tests were performed 24, 48 and 72 hours (Day 4, 5, 6) after the acquisition trial. Each rat was again placed in the illuminated compartment and allowed to step into the dark, preferred chamber. Step-through latency during the procedure was recorded with a 240 s ceiling.

Before the experiment started rats were divided at random into four groups (n = 8–9). Control rats received AS without previous treatment (1^{st} group). IMO+C exposure (60 min) was terminated 120 min before AS delivery (2^{nd} group). SCP was given i.p. 60 min before AS (SCP/60) (3^{rd} group). The fourth group of animals (IMO+C/SCP) received a combination of IMO+C60/120 and SCP/60.

Data were evaluated by one-way ANOVA followed by the Tukey's multiple test with the aim to compare difference between two particular groups ($p < 0.05$). A comparison of avoidance latencies measured on Day 3 and 4 were analyzed by Bonferroni's test.

Fig. 1 summarizes the effect of stress and SCP on the entrance latencies. They decreased during two pre-training days (Day 1 and 2 are not shown). On the training day (Day 3) neither IMO+C60/120 nor SCP/60 alone or their combination changed the entrance latency compared to controls.

The overall analysis revealed a significant difference in the entrance latency measured on Day 4 ($F = 9.04$, $p = 0.0002$), Day 5 ($F = 10.72$, $p < 0.0001$) and Day 6 ($F = 5.64$, $p = 0.003$). On Day 4, a significant increase in avoidance latency was found in all groups (statistical values for controls: $t = 17.9$, $p < 0.001$; for IMO+C60/120: $t = 4.4$, $p < 0.01$; for SCP: $t = 7.7$, $p < 0.001$; for IMO+C/SCP: $t = 3.0$, $p < 0.05$). In all post-training days IMO+C60/120 produced a significant decrease of the entrance latency when compared to the control group. On Day 4 the application of SCP/60 was not different from the control group, however, the following two days SCP/60 induced significant decrease of the latency. The combination of stressor exposure with SCP (IMO+C/SCP) was different not only from controls ($p < 0.01$) but also from SCP/60 on Day 4 and on Day 5. Similar results were obtained on Day 6.

These experiments examined whether the exposure to an acute stressor of restraint and water immersion influenced acquisition and retention in a passive avoidance task and whether this effect was influenced by i.p. application of SCP. Stressor exposure terminating two hours before AS effectively disrupted learning of

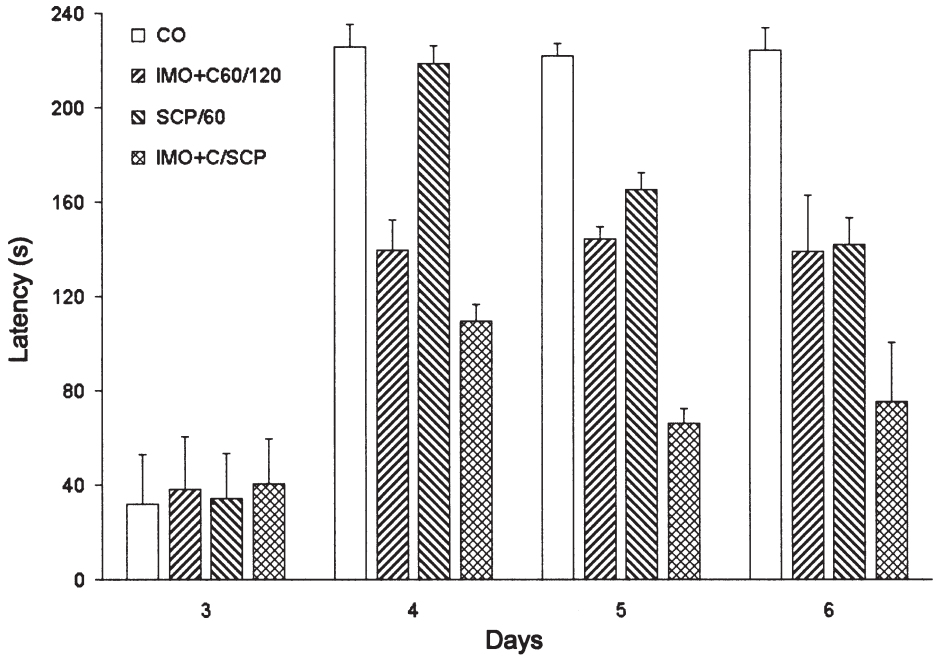

Figure 1. The effect of restraint stress combined with water immersion for 60 min (IMO+C), the effects of i.p. stresscopin (SCP) and their combination on the memory performance of Wistar rats tested by passive avoidance procedure; on day 3 rats received a footshock, and in the following days 4, 5, 6 the retention memory tests were performed (for details see text). Data latencies to enter dark compartment were recorded in seconds (s) with a 240 s ceiling; data are presented as average values ± S.E.M. Statistical evaluation of results is presented in the text.

inhibitory avoidance observed in retention testing 24–72 hours following acquisition. In spite of the fact that SCP as peptide was applied i.p., and that there was a small chance of its full penetration to brain tissues, we have observed its significant effect on rat's behavior in the passive avoidance device. The application of SCP one hour after termination of stressor exposure and one hour before footshock induced impairment of acquisition that was comparable to that induced by the stressor alone. SCP alone did not produce any learning deficit as recorded on Day 4, however, the avoidance latency decreased compared to the control group on Day 5 and 6; this finding implicates that the memory trace under the influence of SCP was not as durable as that formed in control animals. The disruptive effect on memory formation was more profound when SCP was combined with IMO+C. These findings are in contradiction to our expectations because the activation of $CRFR_2$ should ameliorate the stress effects. We have no appropriate explanation for this finding. There exists a possibility that SCP exerts peripheral effects that

may ultimately result in behavioral changes induced by stress. For future studies it would be desirable to test the effects of SCP and related peptides injected *via* cannulae into the specific brain regions.

Acknowledgements. The authors are grateful to RNDr. M. Flegel, CSc. (PolyPeptide Laboratories, Prague, Czech Republic) for kind supply of the sample of stresscopin. This study was supported by grants of IGA Ministry of Health CR No. I-6627-3, and MSM No. 1111 0000 1.

References

Aguilera G. (1998): Corticotropin releasing hormone, receptor regulation and the stress response. Trends Endocrinol. Metab. **9**, 329—336

Chan R. K., Vale W. W., Sawchenko P. E. (2000): Paradoxical activational effects of a corticotropin-releasing factor-binding protein "ligand inhibitor" in rat brain. Neuroscience **101**, 115—129

Coste S. C., Murray S. E., Stenzel-Poore M. P. (2001): Animal models of CRH excess and CRH receptor deficiency display altered adaptations to stress. Peptides **22**, 733—741

Dautzenberg F. M., Hauger R. L. (2002): The CRF peptide family and their receptors: yet more partners discovered. Trends in Pharmacol. Sci. **23**, 71—77

Herman J. P., Cullinan W. E. (1997): Neurocircuitry of stress: central control of hypothalamo-pituitary-adrenocortical axis. Trends in Neurosciences **20**, 78—84

Higelin J., Py-Lang G., Paternoster C., Ellis G. J., Patel A., Dautzenberg F. M. (2001): ^{125}I-Antisauvagine-30: a novel and specific high-affinity radioligand for the characterization of corticotropin-releasing factor type 2 receptors. Neuropharmacology **40**, 114—122

Hsu S. Y., Hsueh A. J. (2001): Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat. Med. **7**, 605—611

Kiss A., Jezova D. (2001): Lesion of central part of the dorsomedial nucleus alters vasopressin but not corticotropin releasing hormone mRNA levels in rat hypothalamic paraventricular nucleus. Gen. Physiol. Biophys. **20**, 393—400

Klenerová V., Kaminský O., Šída P., Krejčí I., Hliňák Z., Hynie S. (2002): Impaired passive avoidance acquisition in Sprague-Dawley and Lewis rats after restraint and cold stress. Behav. Brain Res. **136**, 21—29

Li C., Vaughan J., Sawchenko P. E., Vale W. W. (2002): Urocortin III-immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. J. Neurosci. **22**, 991—1001

Lupien S. J., McEwen B. S. (1997): The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain. Res. Rev. **24**, 1—27

McGaugh J. L., Roozendaal B. (2002): Role of adrenal stress hormones in forming of lasting memories in the brain. Curr. Opin. Neurobiol. **12**, 205—210

Petkova-Kirova P. S., Lubomirov L. T., Gagov H. S., Kolev V. B., Duridanova D. B. (2001): Thyrotropin-releasing hormone activates K_{Ca} channels in gastric smooth muscle cells via intracellular Ca^{2+} release. Gen. Physiol. Biophys. **20**, 43—60

Reul J. M., Holsboer F. (2002): Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr. Opin. Pharmacol. **2**, 23—33

Shi M., Yan X., Ryan D. H., Harris R. B. (2000): Identification of urocortin mRNA antisense transcripts in rat tissue. Brain Res. Bull. **53**, 317—324

Skelton K. H., Owens M. J., Nemeroff C. B. (2000): The neurobiology of urocortin. Reg. Peptides **93**, 85—92

Smagin G. N., Heinrichs S. C., Dunn A. J. (2001): The role of CRH in behavioural responses to stress. Peptides **22**, 713—724

Final version accepted: December 20, 2002