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STIMULATION OF PROTEIN KINASE C REDUCES ACTH-INDUCED EXCESSIVE GROOMING

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Intracerebroventricular (i.c.v.) treatment of rats with melanocortins (ACTH, MSH) results in the display of excessive grooming, interrupted by bouts of stretching and yawning behavior (Gispen and Isaacson, 1981). Changes in the grooming pattern after specific neuropharmacological manipulation of dopaminergic, GABAergic and opiate-related brain systems are apparent in the second part of a one hour observation period immediately following the i.c.v. administration of the peptide (Isaacson et al., 1983). The mechanism by which ACTH interacts with the relevant brain structures to produce grooming is largely unknown. Nonetheless there is increasing evidence that ACTH and congeners may specifically regulate receptor-mediated signal transduction in presynaptic membranes in rat brain by inhibition of protein kinase C (Gispen et al., 1985). Activation of receptors known to mobilize intracellular calcium results in the specific hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) yielding diacylglycerol and inositol 1,4,5,-trisphosphate. The latter is assumed to represent the calcium-mobilizing signal, whereas the former stimulates protein kinase C (Berridge and Irvine, 1985). In synaptic membranes the predominant substrate of protein kinase C is the neural-specific protein B-50 (MW 48 kDa, IEP 4.5). Phosphorylated protein B-50 is an inhibitor of PIP kinase and thus presumably exerts a negative feedback control on the synthesis of PIP_2 (Gispen et al., 1985). In synaptic membranes, melanocortins are known to inhibit protein kinase C, yielding less phosphorylated B-50 and, as a consequence, interrupting the feedback control and making more PIP_2 available for receptor-activated hydrolysis (Gispen et al., 1985). The structure-activity relationship for ACTH-induced grooming in vivo and that for ACTH inhibition of protein kinase C in vitro are almost identical (Gispen and Isaacson, 1981; Gispen et al., 1985). Therefore, it was deemed of interest to study whether ACTH-induced excessive grooming involved protein kinase C. The influence of i.c.v. application of the protein kinase C stimulator 1,2-dioctanoylglycerol (DOG; Lapetina et al., 1985) on ACTH-induced excessive grooming was therefore investigated.

The surgical and behavioral procedures are detailed in Gispen and Isaacson (1981). In short, male Wistar rats (TNO, Zeist, NL) weighing 140–150 g were used. One week prior to the grooming test a polyethylene cannula was implanted into the third brain ventricle. The rats received an i.c.v. injection of either saline or synthetic $ACTH_{1-24}$ (Organon Int. BV, Oss, NL; 0.1 μg/3.0 μl saline at the beginning of the behavioral test. The animals were then placed individually in novel glass boxes and received 10 min after the first i.c.v. injection, a second i.c.v. injection of either 0.5% ethanol in saline (vehicle) or DOG (Avanti Polar Lipids, Birmingham, AL, USA; 1 or 10 μg/3.0 μl vehicle). Observation of grooming behavior began 10 min after the second i.c.v. injection and lasted for 35 min. Every 15th s an observer scored whether the rats displayed elements of the grooming repertoire, yielding a maximal score of 140. The individual grooming scores were averaged per group and analyzed by a one-factor

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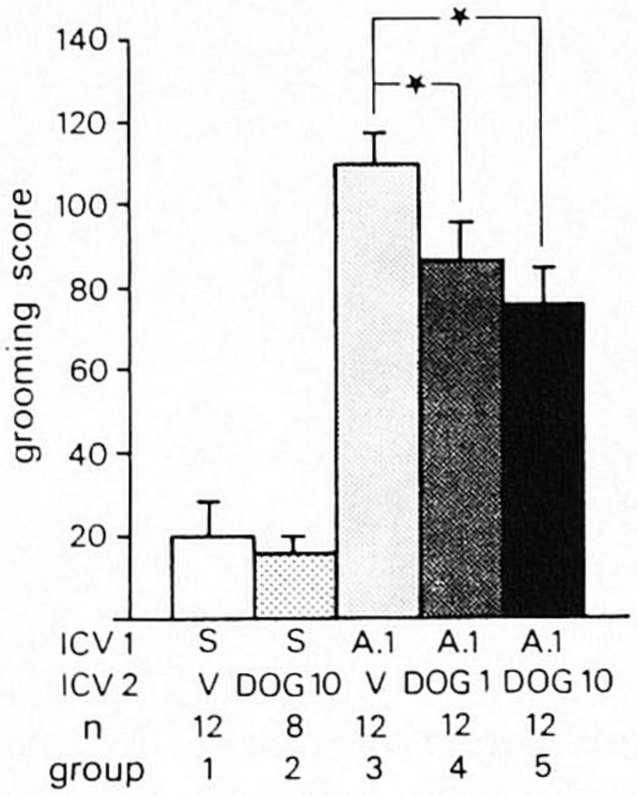

Fig. 1. The effect of 1,2-dioctanoylglycerol on ACTH-induced excessive grooming. ICV 1 and ICV 2: first and second intracerebroventricular injection; S: saline; V: 0.5% ethanol in saline; A.1: 0.1 μg ACTH-(1–24) in saline; DOG 1 or 10: 1 or 10 μg dioctanoylglycerol in 0.5% ethanol in saline; n: number of rats. One-factor ANOVA: $F = 62.3$ df 4/43 supplemental t-tests, two-tailed $P < 0.05$ for differences between group 3 and all other groups; group 2 and groups 4 and 5; group 1 and groups 4 and 5. Bars denote mean $\pm$ S.E.M. *Denotes significant differences between groups 3, 4 and 5.

analysis of variance followed by an adjusted supplemental t-test.

As can be seen in fig. 1, saline/vehicle-treated rats showed little grooming activity during the observation period and were asleep most of the time. In contrast, rats treated with ACTH displayed grooming behavior which lasted for a major part of the observation period (ca. 80% of total possible grooming score). Treatment with DOG did not influence the behavior of saline-treated rats in the novel glass boxes. If, however, the i.c.v. treatment with ACTH-(1–24) was followed by the i.c.v. administration of 1 or 10 μg DOG a significant reduction of the grooming score by 22 and 31%, respectively, was obtained. At the end of the observation period the behavior of these rats was similar to that seen in saline/vehicle- and saline/DOG-treated rats. Hence, the present data are the first evidence suggesting that ACTH may induce excessive grooming by a neurochemical mechanism that involves protein kinase C. Further research is in progress to study the influence of protein kinase C activators on ACTH-modulated behaviors in more detail.

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References

Berridge, M.J. and R.F. Irvine, 1985, Inositol trisphosphate and calcium mobilization, in: Inositol and Phosphoinositides, eds. J.E. Bleasdale, J. Eichberg and G. Hauser (Humana Press, Clifton, NJ, U.S.A.) p. 351.

Gispen, W.H. and R.L. Isaacson, 1981, ACTH-induced excessive grooming in the rat, Pharmacol. Ther. 12, 209.

Gispen, W.H., J.L.M. Leunissen, A.B. Oestreicher, A.J. Verkleij and H. Zwiers, 1985, Presynaptic localization of B-50 phosphoprotein: the (ACTH)-sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism, Brain Res. 328, 381.

Isaacson, R.L., J.H. Hannigan, Jr., J.H. Brakkee and W.H. Gispen, 1983, The time course of excessive grooming after neuropeptide administration, Brain Res. Bull. 11, 289.

Lapetina, E., B. Reep, B.R. Ganong and R.M. Bell, 1985, Exogenous sn-1,2-diacylglycerols containing saturated fatty acids function as bioregulators of protein kinase C in human platelets, J. Biol. Chem. 260, 1358.