

as mean \pm SE of the mean and statistical significance was calculated by Student's t-test.

Results and discussion. The results of the present experiments are summarized in the table. It can be seen that the amount of dopamine in the corpus striatum of rats irradiated with 850 R was significantly decreased on day 1, 2 and 5 following irradiation, as compared with the control values ($p < 0.001$). On the other hand, peripheral application of 1-DOPA and benserazide produced a significant increase in the amount of dopamine in the corpus striatum of irradiated rats regardless of the post-irradiation period investigated ($p < 0.001$). Similarly, pretreatment of nonirradiated control animals with a mixture of 1-DOPA and benserazide resulted 1, 2 and 5 days later in a remarkable rise of dopamine in the corpus striatum ($p < 0.001$). When nonirradiated controls were treated with 100 mg/kg of 1-DOPA only, the dopamine content in the corpus striatum was also higher in all the 3 investigated points than in the nontreated controls ($p < 0.05$). As expected, a significant difference was found between 1-DOPA-treated and 1-DOPA plus benserazide treated nonirradiated controls irrespective of the post-irradiation period studied ($p < 0.001$). The dopamine content was twice as large in the corpus striatum of the latter, due to the protective action of benserazide on 1-DOPA by the inhibition of DOPA-decarboxylase.

Aromatic 1-amino acid decarboxylase can be selectively inhibited in the cerebral and extracerebral tissues by a variety of drugs. For instance, pretreatment with N-(DL-seryl)-N'-(2,3,4-trihydroxybenzyl) hydrazine (Ro-4-4602), DL-amethyl- α -hydrazino-3,4-dihydrophenylpropionic acid (HMD) or NSD 1015 (m-hydroxybenzyl-hydrazine) markedly enhances the DOPA-induced increase of catecholamines in the brain, and to a lesser extent in the liver, heart and kidney¹²⁻¹⁵. The administration of 1-DOPA after inhibition of peripheral DOPA-decarboxylase is thought to induce an increase in brain catecholamines as a consequence of increased penetration of this amino acid into the brain where decarboxylation is taking place.

The above finding that the i.p. injection of 1-DOPA and benserazide significantly increase the content of dopamine in the corpus striatum of rats irradiated with 850 R, as measured 1, 2 and 5 days after irradiation, suggests that these animals are still able to metabolize the catecholamine precursor after peripheral inhibition of 1-DOPA-decarboxylase. We have previously shown that, in rats irradiated

with 650 or 850 R, the application of 1-DOPA and benserazide produced a significant increase in the amount of noradrenaline or dopamine in the heart atria and hypothalamus, as measured 24 h after irradiation, compared to irradiated controls⁵.

The results obtained suggest that precursor uptake and metabolism, and probably amine storage, remained unaltered after irradiation.

- 1 Acknowledgment. We wish to acknowledge the expert technical assistance of Gordana Milenković.
- 2 Stepanović, S.R., Nikolić, J.V., and Konstantinova, M.S., *Yugosl. physiol. pharmac. Acta* 13 (1977) 129.
- 3 Stepanović, S.R., and Nikolić, J.V., *Yugosl. physiol. pharmac. Acta* 14 (1978) 439.
- 4 Stepanović, S.R., and Nikolić, J.V., *Experientia* 35 (1979) 111.
- 5 Stepanović, S.R., Nikolić, J.V., and Varagić, V.M., in: *Catecholamines and stress: recent advances*, p.95. Eds E. Usdin, R. Kvetnansky and I. Kopin. Elsevier, Amsterdam 1980.
- 6 Anderson, C.T., Blaschko, R., Burn, J.H., and Mole, R.H., *Br. J. Pharmac.* 6 (1951) 342.
- 7 Kulinski, V.I., and Semenov, L.F., *Radiobiologia* 5 (1965) 494.
- 8 Dalhström, A., Häggendal, J., and Rosengren, B., *Acta radiol. ther.* 12 (1973) 191.
- 9 Kvetnansky, R., and Mikulaj, L., *Endocrinology* 87 (1970) 738.
- 10 Kvetnansky, R., Sun, C.L., Lake, C.R., Thoa, N., Torda, T., and Kopin, I.J., *Endocrinology* 103 (1978) 1968.
- 11 Kvetnansky, R., Weise, V.K., and Kopin, I.J., in: *Catecholamines: basic and clinical frontiers*, vol. 1, p.684. Eds E. Usdin, I. Kopin and J. Barchas. Pergamon Press, New York 1979.
- 12 Bartholini, G., and Pletscher, A., *J. Pharm. Pharmac.* 21 (1969) 323.
- 13 Lotti, V.J., and Porter, C.C., *J. Pharmac. exp. Ther.* 172 (1970) 406.
- 14 Wurtman, R.J., Larin, F., Mostafapour, S., and Fernstrom, J.D., *Science* 185 (1974) 183.
- 15 Gibson, J., and Wurtman, R.J., *Biochem. Pharmac.* 26 (1977) 1137.
- 16 Manuhin, B.N., Bardiševa, L.V., and Volina, E.V., *Vop. med. Hem.* 21 (1975) 225.
- 17 Carlsson, A., and Waldeck, B., *Acta physiol. scand.* 44 (1958) 293.
- 18 Lavery, R., and Taylor, K.M., *Analyt. Biochem.* 22, (1968) 269.

0014-4754/83/091050-02\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1983

Glyceryl-1,2-dioleate-3-palmitate, a brood pheromone of the honey bee (*Apis mellifera* L.)

N. Koeniger and H. J. Veith

Institut für Bienenkunde (Polytechnische Gesellschaft), Universität Frankfurt, D-6370 Oberursel/Ts. (Federal Republic of Germany), and Institut für Organische Chemie und Biochemie der Technischen Hochschule, D-6100 Darmstadt (Federal Republic of Germany), October 8, 1982

Summary. Glyceryl-1,2-dioleate-3-palmitate, a brood pheromone, was isolated and identified from drone pupae of *Apis mellifera* L. Broodcare is an essential element in the life of the honey bee, but it is still unknown how bees recognize their brood, some aspects of this question are examined in this paper. Glyceryl-1,2-dioleate-3-palmitate, a brood pheromone, was isolated and identified from drone pupae of *Apis mellifera* L.

Material and methods. When 2 samples of brood are offered to a group of hive bees, they choose, settle on and warm only one. Experiments have shown that mechanical and chemical stimuli (demonstrated with ether extracts of worker, queen or drone pupae) are essential for the induction of incubation of brood¹.

A bioassay was used to isolate and identify the biologically active compounds: A group of 400–800 hive bees was confined to a cage (21 \times 12 \times 6 cm). The cover of the cage had 3 openings. The center hole contained a honey feeder and the side holes – 6 cm distant from the central one – were closed by semi-artificial queen cells. They were com-

posed of a natural silk cocoon, which was obtained by thorough extraction of queen cells with diethyl ether.

One of these cocoons was impregnated with an extract of drone pupae or fractions of it, the other (control) with the same amount of solvent.

The test cage was kept in a dark room with a temperature of 15 °C. After 30 min the running activity of the bees decreased and a cluster built up. This process was finished 1 or 2 h after the start of the test.

Then it was noted which cocoon was covered by the bees and integrated into the now well-ordered bee cluster. Tests which resulted in the covering of both or non of the cocoons were rejected.

3000 drone pupae were extracted with 300 ml diethyl ether at room temperature. The crude extract was directly injected on a HPLC column (Perkin-Elmer Series 2, Lichrosorb Si 60, 16 × 250, UV-detector 247 nm, CHCl₃, 17 ml/min) and collected fractions were subjected to the biotest. The biological active fraction was purified by HPLC and analyzed by MS under electron impact and field desorption (FD) ionization conditions (Finnigan MAT 311 A, equipped with a combined FD/FI-EI ion source), by ¹H-NMR (Bruker WM 300, 300 MHz, CDCl₃) and IR-spectroscopy (Perkin-Elmer 355, CHCl₃).

Results. The FD-mass-spectrum of the biological active substance (HPLC retention time 5 min) shows a single peak for the molecular ion at *m/z* 858; high resolution MS establishes the elemental composition C₅₅H₁₀₂O₆ of the molecule. The fragment ions in EJ-MS *m/z* 603, 577, 339, 313, 265, 264 and 239 indicate a triglyceride with 1 palmitate and 2 oleate residues with the palmitate acid likely present in position 3 of the glycerol¹.

The presence of the glycerol unit is definitely identified by the signals (in ppm from TMS) at 5.33 (1 H), 4.25 (2H), while in the IR-spectrum the carbonyl absorption of the

ester functions appears at 1725 cm⁻¹. Based on these results the glyceryl-1,2-dioleate-3-palmitate was synthesized.

The reaction of 25 mg 1,2-dioleine in 40 ml CH₂Cl₂ and 5 mg triethylamine with 11.5 mg palmitoylchloride was catalyzed by 1 mg dimethylamino pyridine². The obtained triglyceride was purified by TLC (silica gel, Merck, CHCl₃, R_F: 0.6) and characterized by spectroscopic methods.

The test cocoon was impregnated with 10–20 µl ether extract containing the equivalent of 5–12 drone pupae and the control cocoons with the same amount of ether. The test showed that 72 times the test cells were more attractive compared to 12 times for the controls.

The HPLC fraction (retention time 5 min) gave a proportion of 65 (fraction) to 11 (control). The synthetic glyceryl-1,2-dioleate-3-palmitate (7 µg) was also offered on the test cocoon, resulting in 32 (test) to 12 (control). Due to the results of chemical analyses we suspected that olive oil contained the active substance also.

So the test cocoons were impregnated with 0.4 µl olive oil. The results were 34 (oil) to 6 (controls). All ratios reported here, are at least significant on a 1% level (χ²).

Experiments on other substances and a more detailed discussion will be published elsewhere.

- 1 Koeniger, N., *Apidologie* 9 (1978) 305.
- 2 Odham, G., and Stenhagen, E., in: *Biochemical applications of mass spectrometry*, p.229. Ed. G.R. Waller. Wiley, New York 1972.
- 3 Höfle, G., Steglich, W., and Vorbrüggen, H., *Angew. Chem.* 90 (1978) 602; *Angew. Chem. int. edn. Engl.* 17 (1978) 569.

0014-4754/83/091051-02\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1983

Preparative electrophoresis for large scale preparation of highly purified bovine growth hormone¹

C. Secchi, P. A. Biondi, A. Berrini and S. Ronchi

Institute of Veterinary Physiology and Biochemistry, University of Milan, Via Celoria 10, I-20133 Milan (Italy), October 19, 1982

Summary. A method allowing large scale preparation of bovine growth hormone from pituitary glands in relatively few steps is described. In comparison to conventional purification techniques previously reported, the use of preparative polyacrylamide gel electrophoresis reduces the number of steps and the amount of time needed for the isolation of the hormone. In addition, the yield is several times greater, the hormone is purer and it has greater bioactivity (1.83 IU/mg).

Bovine growth hormone² is a single polypeptide chain consisting of 191 residues, with a known sequence^{3–5}. The availability of large amounts of homogeneous bGH might be useful for studies on regulation of bGH secretion or for clinical use. To date, few bGH isolation procedures have employed preparative electrophoresis^{6,7}.

In view of the shortcomings of classical methods of hormone fractionation⁸, the present investigation, involving the use of a rapid preparative polyacrylamide gel electrophoresis (Prep-PAGE), was developed. In the apparatus used, a slab shaped gel is cast directly between 2 cooled glass surfaces. The major problem associated with the Prep-PAGE technique, inefficiency of heat dissipation, is overcome by making the gel with an enlarged surface area. The apparatus also adopts a symmetrical elution chamber in which the buffer enters equally from all points, which results in a ready elution of separated components. Protein better than 90% pure and free of detergents, urea or carrier

ampholytes were recovered with 73% efficiency. The procedure was also directly applicable to preparation of a product for lyophilization.

Materials and methods. Reagents. Acrylamide and N,N'-methylene-bis-acrylamide of commercial grade were recrystallized from chloroform and acetone respectively. Coomassie Brilliant Blue R-250, silver nitrate and other chemicals were of analytical grade (all obtained from Serva Heidelberg, FRG). Carrier Ampholines of various pH range were purchased from LKB (Bromma, Sweden) and the low molecular weight calibration kit for SDS-PAGE (range 84,000–14,300 i.e. phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme) from Bio-Rad Laboratories (Richmond, CA).

Hormone preparation. The starting material was prepared as previously described using the alternative method of precipitating the crude extract by ammonium sulfate at