amino acid (figure A), whereas in spinal cord cultures only few neurones were labelled (figure B). Figures C and D illustrate a neurone of a brain stem and a spinal cord culture with an intense labelling over the cell body and processes. Other neurones (arrows) in the same cultures were almost free of label. In contrast, in cultures of cerebellum and DRG, ^{3}H-β-alanine was only taken up by glial cells. Similar observations have been made in slices of cerebellum and in isolated DRG of the rat, demonstrating that β-alanine was exclusively accumulated by glial cells[10,11]. The time course of the uptake of ^{3}H-β-alanine into glial cells was slower than that of other amino acid transmitters such as glycine, GABA, glutamate and aspartate[15]. After an incubation time of 5 min, glial cells were only weakly labelled with ^{3}H-β-alanine (figure E), whereas after 10 min there was a heavy accumulation of the amino acid in all glial cells (figure F). These observations are consistent with biochemical studies in the frog spinal cord[7] and in brain slices of the rat[8], demonstrating a slower rate of uptake of β-alanine in comparison to the rapid time course of GABA. The uptake of ^{3}H-β-alanine was temperature- and sodium-dependent being considerably reduced or abolished after incubation at 0 °C or in sodium-free incubation medium. Our results, in demonstrating that in cultured spinal cord and brain stem ^{3}H-β-alanine is taken up by neurones and not only by glial cells, provide further evidence for a transmitter role of β-alanine in these regions[1].

1 F.V. DeFeudis and R. Martin del Rio, Gen. Pharmac. *8*, 177 (1977).
2 R. Martin del Rio, L.M. Orensanz-Muñoz and F.V. DeFeudis, Exp. Brain Res. *28*, 225 (1977).
3 D.R. Curtis, L. Hösli, G.A.R. Johnston and I.H. Johnston, Exp. Brain Res. *5*, 235 (1968).
4 L. Hösli and A.K. Tebēcis, 5 D.R. Curtis and G.A.R. Johnston, Rev. Physiol. Biochem. Pharmac. *69*, 97 (1974).
6 K. Krnjević, Physiol. Rev. *54*, 418 (1974).
7 R. Adair and R.A. Davidoff, J. Neurochem. *29*, 213 (1977).
8 D.R. Riddall, M.J. Leach and A.N. Davison, J. Neurochem. *27*, 835 (1976).
9 D. Lodge, G.A.R. Johnston and A.L. Stephenson, J. Neurochem. *27*, 1569 (1976).
10 F. Schon and J.S. Kelly, Brain Res. *86*, 243 (1975).
11 J.S. Kelly and F. Dick, Cold Spring Harb. Symp. quant. Biol. *40*, 93 (1976).
12 A. Bruun, B. Ehinger and A. Forsberg, Exp. Brain Res. *19*, 239 (1974).
13 L. Hösli, E. Hösli, P.F. Andrès and J.R. Wolff, in: Golgi Centennial Symposium. Proc., p.473. Ed. M. Santini. Raven Press, New York 1975.
14 E.C. Jenkins, Stain Technol. *47*, 23 (1972).
15 L. Hösli and E. Hösli, Rev. Physiol. Biochem. Pharmac. *81*, 135 (1978).

Exogenous melatonin and melanophore development in *Xenopus*

P.C. Baker, K.M. Hoff and R.E. Buda

Department of Biology, Cleveland State University, Cleveland (Ohio 44115 USA), 10 April 1978

Summary. *Xenopus* larvae raised from stage 21 in melatonin solution and upon a dark background had fewer head melanophores at stage 48 than control animals not exposed to melatonin. Rearing larvae in melatonin solution seems to mimic rearing larvae on a light background.

The ability to mimic the body blanching response of *Xenopus* larvae with the pineal substance melatonin provides not only an extremely specific bioassay method[1]; but serves as the basis for a well defined theory of pineal control of body blanching in this species[2]. Placing larvae on a white background will also produce blanching[3], and rearing *Xenopus* embryos on a white background will even reduce the number of melanophores present in the larvae[4,5] We have reared *Xenopus* embryos in melatonin solution to see whether melanophore number is changed and we find that it is, although exposure to melatonin must occur early in development.

Methods. *Xenopus laevis* embryos were obtained by artificial ovulation and breeding, and staged using the normal table of Nieuwhoop and Faber[6]. At stage 20 embryos were removed from their investing membranes with sharpened jeweler's forceps and transferred to appropriate dishes. Embryos were cultured in groups of 10 in covered finger bowls on a black background containing 50 ml spring water or 50 ml spring water with a melatonin concentration of 2 µg/ml. During development the water was changed periodically. Embryos were introduced to the melatonin solution at stages 21, 26, 35/36 and 40. Embryos were killed at stage 48 by transferring to neutral buffered formalin solution and melanophores on the right side of the head were counted[5].

Results. Only the embryos introduced to the melatonin solution at stage 21 showed any significant difference from spring water control larvae. Their melanophore number was only 58% of control with a SEM of 4.9% and a $p < 0.001$ derived from Student's t-test.

Discussion. The significant difference related to melanophores between stage 21 embryos and later stages tested is the migration of neural crest tissue, although definitive differentiation into melanophores is not manifested until stage 33/34[6]. Previous measurement of light and dark background reared embryos showed a melanophore reduction in light background animals that averaged about 59% of dark background animals[5]. The involvement of endogenous melatonin in melanophore development is difficult however to ascertain. Melatonin level is not in any way outstanding at stage 20[7], nor is its formative enzyme hydroxindole-O-methyltransferase (HIOMT)[8]. In animals reared on differing backgrounds and then measured for melatonin and HIOMT at stage 48 there is a reduction of melatonin levels in light background reared larvae of about one half[5] and an increase in whole embryo HIOMT of about 40%[9]. The HIOMT increase is centered in the brain where a 9fold elevation of light background over dark background is found[9]. Although melatonin has not been implicated in developmental processes as has its precursor 5-hydroxytryptamine (5-HT)[10], it has been suggested as a possible agent in cellular contractile phenomena[11]. While there is no suggestable mechanism of action for the effect of exogenous melatonin upon melanophore development, it is of interest to note that it can be used to mimic light background rearing just as it can be used to mimic body blanching.

1 W.B. Quay and J.T. Bagnara, Archs Pharmacodyn. *150*, 137 (1964).
2 J.T. Bagnara, Gen. Comp. Endocr. *3*, 86 (1963).
3 J.T. Bagnara and M.E. Hadley, Am. Zool. *10*, 201 (1970).
4 F.W. Pehlemann, Zool. Anz., suppl., *29*, 571 (1967).
5 P.C. Baker and K.M. Hoff, Comp. gen. Pharmac. *2*, 59 (1971).
6 P.D. Nieuwhoop and J. Faber, in: Normal Table of Xenopus laevis. North Holland Publishing Company, Amsterdam 1956.
7 P.C. Baker, Comp. Biochem. Physiol. *28*, 1387 (1969).
8 P.C. Baker, W.B. Quay and J. Axelrod, Life Sci. *4*, 1981 (1965).
9 P.C. Baker, K.M. Hoff and R.L. Clise, Comp. gen. Pharmac. *2*, 397 (1971).
10 G.A. Buznikov, in: Comparative Pharmacology, vol. I, p. 593. Ed. M.J. Michelson. Pergamon Press, Oxford 1973.
11 W.B. Quay, Adv. Pharmac. *6A*, 283 (1968).

The lack of an effect of cholinergic agonists on anterior pituitary prolactin production in vitro[1]

M.D. Campbell, S. Jaques, Jr, and R.R. Gala[2]

Department of Physiology, Wayne State University School of Medicine, Detroit (Michigan 48201, USA), 8 March 1978

Summary. The addition of dopamine to anterior pituitary incubations resulted in a marked decrease (88% for ^{3}H prolactin and 69% for RIA prolactin) in prolactin release. Incubation with the cholinergic agonists carbacol, arecoline and nicotine resulted in no significant change in prolactin secretion.

The role of the cholinergic system in the regulation of prolactin secretion is not well understood. Subramanian and Gala[3] have shown that cholinergic agonists can inhibit the afternoon surge of prolactin when administered systemically to ovariectomized estrogen-treated rats. Lawson and Gala[4], however, have shown that systemically administered cholinergic agonists had little effect on basal prolactin levels in ovariectomized estrogen-treated rats. Vale et al.[5] have suggested that the pituitary may have cholinergic receptors since the addition of carbacol to anterior pituitary cell cultures and explants resulted in an inhibition of prolactin secretion. The purpose of the present study was to determine the effects of a muscarinic agonist, arecoline, a nicotinic agonist, nicotine, and carbacol, a mixed cholinergic agonist on prolactin secretion by anterior pituitary (AP) explants.

Materials and methods. Adult female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) weighing approximately 250 g were housed 2 per cage, and placed on a 14:10 h lighting schedule. All animals had free access to water and Purina Rat Chow. Approximately 5 days later animals were decapitated, the AP quickly excised, and dissected into 4 or 6 pieces. An AP fragment from each pituitary was placed in each group so as to permit statistical analysis with a paired t-test. Each experiment had a total of either 4 or 6 vials per group and each vial contained 1 AP equivalent. Pituitaries were incubated as described previously by MacLeod and Lehmeyer[6] with the following modifications. The AP were preincubated for 2 h in 1 ml Hanks' balanced salt solution containing 10 µCi 4,5-^{3}H-leucine/ml. The pituitaries were removed, rinsed with Medium 199 and incubated in Medium 199 for 4 h under a gas environment of 95% O_2-5% CO_2. The incubation medium contained one of the following drugs: dopamine (1.0×10^{-6} M), carbacol (1.0×10^{-5} and 1.0×10^{-4} M), arecoline (1.0×10^{-5} and 1.0×10^{-4} M) and nicotine (1.0×10^{-5} and 1.0×10^{-4} M). At the end of 4 h the AP were removed from the incubation medium and homogenized in 3 ml of a 1% Triton X-100-PBS solution. The resulting solution was centrifuged; the supernatant and the incubation medium were frozen for future assay. The prolactin concentration of the medium and the AP homogenates was determined using a rat prolactin RIA as previously described[7]. The incorporation of 4,5-^{3}H-leucine into prolactin was determined by disc gel electrophoresis[8]. 200 µl of incubation medium and AP homogenate solution were assayed for prolactin using a 7.5% polyacrylamide gel. The stained band was cut from the gel, dissolved in 0.5 ml of NCS solution and the radioactivity counted in a Packard tri-carb spectrophotometer. Statistical analysis of the data was accomplished using the paired t-test and the data is presented as the average ± SEM.

Results. As shown in figures 1 and 2, the addition of dopamine (10^{-6} M) to the incubation medium resulted in a decrease in prolactin release of 88% and 69% as measured by ^{3}H-leucine incorporation into prolactin and RIA, respectively. The decrease in medium prolactin was reflected by an increase in pituitary content for both methods of assay but only ^{3}H prolactin was statistically significant. The addition of cholinergic agonists to the incubation medium

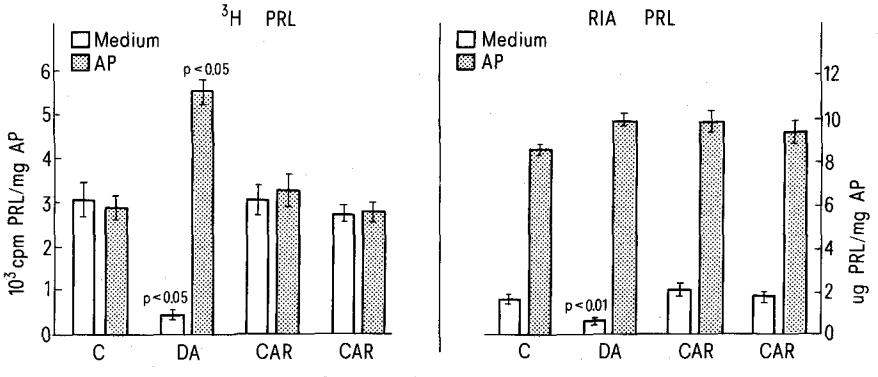

Fig. 1. Effect of dopamine (10^{-6} M) and carbacol (10^{-5} and 10^{-4} M) on prolactin (PRL) secretion by anterior pituitary (AP) explants incubated for 4 h. Each group contained 4 vials and each vial contained 4 fragments representing 1 AP equivalent. The incorporation of 4,5-^{3}H-leucine into prolactin is presented on the left side while RIA prolactin is presented on the right side. Each bar represents the mean ± SEM. C, control; DA, dopamine; CAR, carbacol.