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ABSTRACTS

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RELIABLE METHOD FOR REPEATED BLOOD SAMPLING
HORMONAL ASSAY IN RATS. L. O'Farrell, F.C. Martin,
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Chronic blood sampling is useful for hormonal and pharmacokinetic studies. Blood cannot be sampled past 2 weeks on most cannulation methods, however, and chronic stress as reflected by changes in plasma hormones often occurs. We therefore modified the jugular cannulation technique of F. J. Smith and L. A. Campfield (Am J Phys. 251, R70, 1986) to improve blood sampling reliability, decrease time required for surgery and reduce stress. Cannulae were made from 3 pieces of tubing: 2.3 cm of 1-100 tubing pulled over a flame at one end to an O.D. of .65 mm; 5 cm of .3 mm I.D. by .64 mm O.D. Dow Corning Silastic cut on a level at one end; and 1.5 cm of .76 mm I.D. by 1.65 mm O.D. Silastic. Four mm of the small end of the PE tubing was inserted into a lumen of the non-beveled end of the smaller diameter Silastic. After securing this junction with a piece of suture, the larger diameter Silastic was slid over the smaller and pushed over the junction. Under aseptic conditions, the beveled end of the smaller diameter Silastic was inserted 32 mm into a small incision in the right jugular vein made 5 mm from where the vein enters the chest muscle. The cannula was tied to the surrounding tissue with a suture secured to the cannula with Silastic Elastomer. The other end of the cannula was sealed s.c. to an incision between the scapulae. The cannula was secured to the underlying muscle using a suture secured to the larger diameter Silastic, and the skin was closed with a purse string suture. Cannulae were flushed daily with 50 U/ml heparinized saline mixed with 25 mg/ml cephalosporin. Twenty-one of 24 cannulae implanted in this way remained patent for 1.5 months. Four features of this method were shown to be critical to long-term sampling: the placement of the cannula tip at the junction of the superior vena cava and the atrium (which the method described insures for rats 250-400 gm); the small size of the tubing in the vein; the aseptic surgical technique; and the use of antibiotics. Lights-off plasma testosterone and prolactin levels were 174 ng/ml and 23 ng/ml 1.5 weeks postoperatively, indicating this technique is minimally stressful and suitable for measurement of stress-related hormones. Supported by the David Murdock Foundation for Advanced Brain Research and NIH grant NS07628).

115.10 NEUROCHEMICAL MECHANISMS OF REPRODUCTION IN THE JAPANESE QUAIL :
I. THE PREOPTIC AREA. J. Balthazart (1)*, G.F. Ball (2), M. Gahr (3)* and B.S. McEwen (2) (SPON : ENA).

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The activational effects of testosterone (T) on copulation are sexually differentiated in quail : T activates copulation in males but not in females. We previously identified several neurochemical correlates of this behavioral dimorphism. The inducibility of the preoptic aromatase by T is significantly higher in males than in females (Brain Res. 1986, 370, p285) and the concentration of norepinephrine in the preoptic area (POA) is higher in females than in males (Poultry Sci. 1986, 65, p1413).

To determine more precisely the neuroanatomical localizations of these sexually differentiated aspects of brain biochemistry, we have used immunocytochemistry and quantitative autoradiography to map estrogen receptors on one hand and adrenergic and muscarinic receptors on the other hand. Immunocytochemical mapping using the antibody H222SPgamma raised against E2 receptors from mammary tumors demonstrated the presence of E2 receptors in the POA and especially in the medial preoptic nucleus (POM). Presence of E2 receptors in POM is especially significant as this nucleus is sexually differentiated (Volume larger in males than females; Neurosci. Ltrs. 1986, 64, p129), its volume and coloration by Nissl stains are testosterone-sensitive (decrease by castration and increase following T treatment; Brain Res. 1987 in press) and in addition, POM contains most of the preoptic aromatase as demonstrated by the Palkovits punch technique combined with in vitro radioenzymassays (Brain Res. 1987, in press).

Quantitative autoradiography demonstrated the presence of specific and saturable binding for alpha-2-adrenergic and for muscarinic ligands (respectively tritiated para-amino-clonidine, PAC and n-methyl-scopolamine, NMS). PAC binding was very high in all POA, the highest concentrations of receptors being measured in the POM. Interestingly, PAC binding permitted to outline the area of the POM. This adrenergic receptor type is thus a specific marker for the sexually differentiated nucleus. By contrast, NMS binding was extremely low throughout the POA demonstrating the nearly total absence of muscarinic transmission in this area.

The coexistence in the sexually differentiated POM of E2 and alpha-2-adrenergic receptors suggests that this nucleus is the site of neurochemical interactions which are probably playing a key role in the control of copulation.

115.12 INCREASE OF CIRCULATING LEVELS OF GLUCOSE AFTER THE ELECTROLYTIC LESION OF THE ARCUATE HYPOTHALAMIC NUCLEUS.
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It is well known that the arcuate nucleus of the hypothalamus (ARH) shows specific chemical characteristics, and previous results obtained in our Laboratory suggest a role for this nucleus in the regulation of the blood glucose. To further define this participation we examined the effects of electrolytic lesions of the ARH on the circulating levels of glucose.

Anesthetized male Wistar rats (250gr. body weight) 16 hrs. food deprived were stereotaxically implanted, bilaterally, in ARH with a bipolar concentric electrode. A 2 milliAmp. direct current was applied during 30 sec. in experimental animals. In control rats, no current was applied. Serum glucose was determined before and after lesioning or placing of the electrode.

Subsequent histological analysis revealed that the lesions were confined almost exclusively in the ARH nuclei. Circulating glucose was increases about 130 % after the lesions.

The results obtained suggest that the ARH nucleus participates in the regulation of the glucose. This effect could be mediated by central or peripheral neural connections or by endocrine mechanisms.

NEUROENDOCRINE MECHANISMS OF REPRODUCTION IN THE JAPANESE QUAIL
II: HYPOTHALAMIC AND EXTRA-HYPOTHALAMIC AREAS. M. Gahr*, G.F. Ball, J. Balthazart*, and B.S. McEwen. (SPON: P. Marler) University of Kaiserslautern, Kaiserslautern, F.R.G. The Rockefeller Univ., N.Y., N.Y. 10021, and University of Liège, Liège, Belgium.

The neuroendocrine control of reproduction involves the interaction of steroids and a variety of neurochemical substances in discrete regions of the brain. In the Japanese Quail, the sexually dimorphic pre-optic medial nucleus (POM) has been the focus of recent investigations of the central action of steroids underlying reproductive behavior. In order to better specify other potentially significant sites of steroid-neurotransmitter interactions involved in the control of reproductive processes we have mapped using immunohistochemistry, the distribution of estradiol (E₂) receptors and using quantitative autoradiography the distribution of alpha₁ and alpha₂ adrenergic receptors, and muscarinic cholinergic receptors.

Subjects were male and female Japanese Quail. For autoradiography, frozen brain sections were taken on a cryostat and freeze-dried. Alpha₁ adrenergic receptors were labeled with (³H) prazosin (PRA), alpha₂ adrenergic receptors were labeled with (³H) p-amino-clonidine, and muscarinic cholinergic receptors were labeled with (³H) N-methyl scopolamine. Autoradiograms were analyzed with an image analysis system that converted optical densities to fmoles/mg protein using a standard curve derived from co-exposed plastic standards. E₂ receptors were visualized using the PAP immunohistochemistry technique. A monoclonal antibody (H-222SPY) raised against E₂ receptors was employed.

E₂ receptors were seen in the lateral septum (LS), the nucleus intercollicularis (ICo), and in sub-regions of both the hypothalamus and the archistriatum. Heavy PAC binding was often apparent in regions that contained E₂ receptors. This was particularly striking in certain areas that may play a role in reproduction such as the LS, and the tubero-infundibulum area of the hypothalamus and an area important in vocal behavior, ICo. The distribution of PRA binding was quite different from PAC binding in that the heaviest regions were the dorsolateral cortex (CDL), and portions of the thalamus. NMS binding was highest in the Lobus parolfactorius and area archistriatalis. Other areas showing specific NMS binding include steroid sensitive areas such as the ventro-medial nucleus of the hypothalamus (VMN) and the ICo as well as several non-steroid sensitive areas. The co-occurrence of apparent alpha-adrenergic binding, muscarinic cholinergic binding, and E₂ receptors in a variety of discrete areas implicated in reproduction suggests that steroid interactions with these neurotransmitters may play an important role in the regulation of reproductive processes, including behavior.