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Sex comparisons in doxorubicin-induced muscle and liver damage

T. Phillips, Y.M. Jang and C. Leeuwenburgh

University of Florida, Biochemistry of Aging Laboratory, College of Health and Human Performance, PO Box 118206, Gainesville, FL 32611, USA

Doxorubicin is a member of the anthracycline family of chemotherapeutic agents used to treat many forms of cancer, although its clinical application has been limited due to the cardiotoxicity also induced by the treatment (Childs *et al.* 2002). Few investigations have examined the responses between males and females to doxorubicin therapy in terms of systemic indicators of damage. Thus in this study we examined the responses of male and female Fischer 344 rats at 1 and 4 days following administration of a single dose of doxorubicin (10 mg kg^{-1}). The University of Florida Institutional Animal Care and Use Committee approved all procedures prior to the onset of the study. Doxorubicin or saline was administered intraperitoneally. One or four days later, animals were anaaesthetized with an intraperitoneal injection of sodium pentobarbital (5 mg per 100 g body weight). Blood was collected into Vacutainer tubes containing ethylenediaminetetraacetic acid (K_3EDTA; 8.4 mg per Vacutainer). Liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) were assayed in the plasma as markers of liver damage. Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) levels were measured to evaluate muscle damage. As hypothesized, doxorubicin administration resulted in elevated liver enzyme activity compared with control animals in both males and females. To our surprise, we found no elevation in the muscle damage marker, CK, with the treatment. However, CK activities were significantly lower ($P = 0.007$) in females compared to males. A similar but non-significant trend was also apparent for LDH levels ($P = 0.057$). The dose of doxorubicin administered may have failed to inflict myocardial damage, but was sufficient to impose an insult on liver function. In summary, these findings demonstrate first, a sex difference in CK and LDH activities; and also reveal the influence of doxorubicin on liver function highlighted by the elevations in AST and ALT activities, which appear to be independent of sex.

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All procedures accord with current National guidelines.

PC2

NOS in the cephalopod 'cerebellum'

Anna Di Cosmo*, Carlo Di Cristo* and John B. Messenger†

**Department of Biological and Environmental Sciences, University of Sannio, Benevento, Italy and †Department of Zoology, University of Cambridge, Cambridge, UK*

The brain of *Sepia* is essentially organized hierarchically: motor programmes, usually originating in the optic lobes, are executed via higher motor centres, where specific motor commands are generated and directed to the appropriate sets of motoneurons in the lower centres (Messenger, 1983).

Nitric oxide synthase (NOS) has been shown by immunohistochemistry to be present in specific regions of the central nervous system (CNS) of the cephalopod mollusc *Sepia officinalis* (cuttlefish). NOS activity is Ca^{2+}/calmodulin-dependent and the purified NOS from brain and optic lobes exhibited on SDS-PAGE a band at 150 kDa (Palumbo *et al.* 1999; Di Cosmo *et al.* 2000).

Our finding, that in *Sepia* NOS occurs in higher motor centres, in particular in the spines of the *anterior basal and peduncle lobes*, is most arresting. In both these lobes (studies performed on five animals) there are immunopositive cells of varying sizes, but more importantly there are positively stained fibres in the neuropil. In the posterior anterior basal lobe, the spine region contains numerous immunopositive small cells and strongly staining fine parallel fibres. The fibres run laterally for varying distances along the spine, giving off collateral branches. They presumably serve to carry signals for shorter and longer distances across the lobe. Other immunopositive fibres are seen dorsal and ventral to the immunopositive spine region. Presumably these come from the peduncle lobe. In these areas there are no immunopositive cells. In the peduncle lobe there are a few weakly staining cell bodies. They are seen only in the spine region, lying next to the well-defined boundary of the spine; they are more obvious in the median bank. They are about 10 μm in diameter and sometimes their immunopositive axons are seen entering the spine neuropil. From the peduncle lobe, some immunopositive tracts reach the anterior basal lobe.

In this our findings agree completely with those of Chichery & Chichery (1994), who stained sections of cuttlefish brains for NADPH-diaphorase. Like us they report very strong staining in the posterior anterior basal lobe spine and some staining in the peduncle lobe spine.

These two regions of the brain are thought to constitute cerebellar analogues (Messenger, 1967*a*,*b*; Hobbs & Young, 1973; Messenger, 1979; Camm *et al.* 1985; Gleadall, 1990). Each spine region is characterized by an array of fine parallel fibres intrinsic to the lobe, giving it the appearance of a single folium of a vertebrate cerebellum. In *Octopus* removal of the peduncle lobes does not abolish locomotion, but it does lead to motor dysfunction: movements are imprecise and jerky (Messenger, 1967*b*). Input to these lobes derives from the statocysts, the optic lobes and receptors in the arms and mantle; the output is to lower and intermediate motor centers and to the optic lobes. In short, these lobes are strikingly similar in their function, cyto-architecture and connectivity to the vertebrate cerebellum and, like the cerebellum, which contains the highest level of NOS in the mammalian brain (Rodrigo *et al.* 1994), they are now shown to contain high levels of NOS.

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