300

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AGE-RELATED CHANGES IN P2X3 RECEPTOR EXPRESSION

Aims of Study

For a long time urinary incontinence (UI) was perceived as a problem of the older generation. However, recent studies have shown that it affects 14% of all women over the age of 30 years (Brocklehurst *et al.,* 1993). This figure increases with ageing (Robinson *et al.,* 1997). Urge and mixed incontinence is commoner in older women (Royal College of Physicians, 1995). The pathophysiology of UI is undetermined, but it may be as a result of altered sensory neuromodulation. ATP appears to have an important role in the processing of physiological information in the sensory system. These effects are likely to be mediated by P2X receptors, namely $P2X_3$ (Elneil at al., 2001). How ageing affects $P2X_3$ receptor distribution and function remains to be determined.

In this study we aim to substantiate the distribution pattern of $P2X_3$ receptors in the urothelium and detrusor muscle of the urinary bladder by using Western analysis and also determine whether the expression of $P2X_3$ receptors varied with age. The objective was to see if any changes could be observed in the immunoreactive profile for the receptor subunits as determined by polyacrylamide gel electrophoresis. An electrophoretic mobility shift for a given protein can reflect differences in its post-translational modifications, shown to be critical for the functional activity of many ion channels including members of the P2X receptor family (Nicke *et al*., 1997).

Methods

Membrane fractions were prepared from the urothelium and detrusor muscle of adult (15 - 20 weeks) and elderly (18 - 24 months) Wistar rat urinary bladders, as well as from the transfected clonal cell lines expressing the human recombinant P2X receptor types. Western analysis was carried out on the protein obtained to determine whether $P2X_3$ receptor expression was altered. The Western blots obtained are representative of at least three separate experiments and each panel is taken from a single immunoblot.

Results

Membrane protein extracted from elderly rat bladder urothelium showed a dose-dependent immunoreactivity when analysed by the anti-$P2X_3$ receptor antibody in Western blotting (Figure 1). Two immunoreactive bands were evident with similar intensities, which had apparent molecular masses of 48 and 66 kDa. This immunoreactivity could be abolished by pre-absorbing the antibody to the antigenic peptide to which it had been raised (Figure 1). In younger animals, a similar protein loading gave an intense band at 66 kDa whereas the lower molecular weight species observed in the older tissue was not apparent (Figure 1). In the detrusor muscle extract from both adult and old rats, no immunoreactive staining could be observed unless high concentrations of antibody were used (Figure 1).

$P2X_3$ receptors identified in rat urinary bladder tissue were expressed in two distinct forms. The apparent difference in the size of these proteins can be accounted for by the electrophoretic mobility shifts known to accompany covalent attachment of either phosphate moieties or carbohydrate chains. The urothelial $P2X_3$ receptor pattern appears to exist as a single molecular weight entity in younger animals, and in two forms in older tissue. To determine if the retarded species was the glycosylated form of the protein with higher molecular weight, membrane samples were subjected to deglycosylation. The immunoreactive band of 66 kDa observed in untreated urothelial samples from old rats was only just detected following deglycosylation. In addition, the immunoreactivity of the 48 kDa protein appeared to increase following treatment. For urothelial samples from younger rats, deglycosylation of the 66 kDa product produced several bands with apparent molecular masses ranging between 66 and 48 kDa.

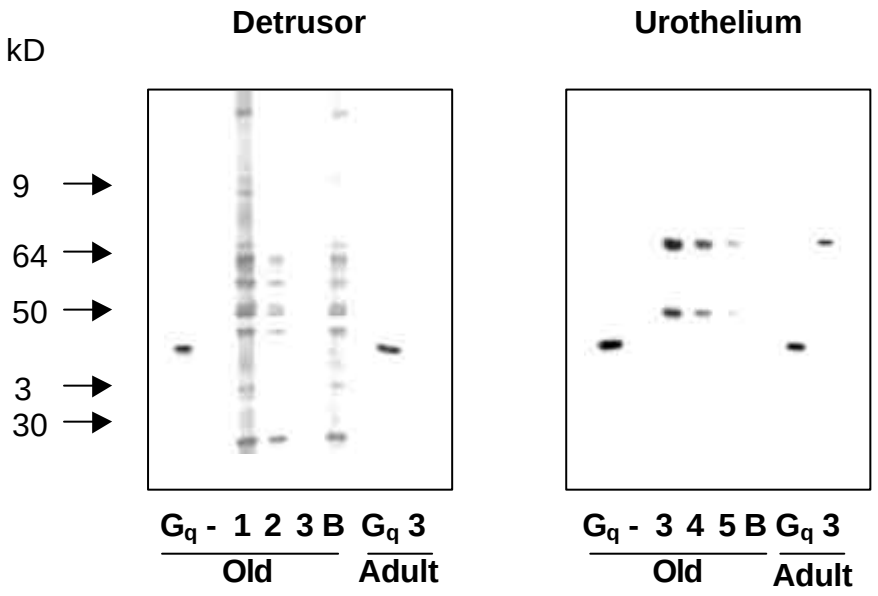

Figure 1: Detection of $P2X_3$ receptor protein in adult and old rat urinary bladder detrusor muscle and urothelium using Western analysis.

Conclusion

The immunoreactive profile for the $P2X_3$ receptor in the urothelium of old rats was in marked contrast to that observed following Western analysis of membrane samples from adult animals. These changes appear to be dependent on the glycosylation state of the receptor. These changes in status of the $P2X_3$ receptor on ageing could have important implications for the functionality of this ion channel and ultimately for the stability of the urinary bladder. binding, cell surface expression, and/or direct channel function.

References

Brocklehurst et al. (1993) *BMJ* **306,** 832-834; Elneil et al (2001) *Pharmacol*, **63**(2):120-8.; Nicke et al. (1997) *Naunyn-Schmiedebergs Arch Pharmacol.,* **355**, Supplement pp. 106S; Robinson et al (1997) *Curr Opin Obs Gyn,* **9,** 285-285.; Royal College of Physicians (1995) Report: *Urinary incontinence.*