stomach lysates (Fig. 2A). Mass spectroscopy was performed to identify exactly the detected bands. The vitellogenin antibody recognized the following proteins: uncharacterized protein at 200 kDa and vitellogenin at approximately 180 and 150 kDa (Fig. 2C). In hemolymph, an additional band was detected. Mass spectroscopy demonstrated that this band belongs to 6-phosphofructokinase, alpha-glucosidase, aconitate hydratase and transferrin. Which of these proteins was recognized by the antibody remains un-identified. Details of the mass spectroscopy analysis are presented in Table 1.

Induction of vitellogenin by clothianidin

As a proof of concept, we determined whether clothianidin leads to induction of vitellogenin. We previously showed that exposure of honey bee workers to clothianidin and additional neonicotinoids led to induction of *vitellogenin* mRNA in the brain (Christen et al. 2016). Here we extended our analysis onto the protein level and aimed to assess the induction of the vitellogenin protein. By Western Blot analysis we quantified vitellogenin in the hemolymph, brain and fat body of bees exposed to 3 ng/bee clothianidin for 24 h. In the hemolymph, vitellogenin levels (full-length and smaller vitellogenin) did not differ between solvent control and clothianidin exposed bees (Fig. 3A). In the brain, exposed bees showed no alteration of the full-length vitellogenin, but the smaller vitellogenin was significantly increased (Figs. 3B, C). In the fat body, a three-fold increase in the amount of full-length vitellogenin occurred in clothianidin exposed bees compared to control bees (Fig. 3D, E). These data indicate that clothianidin led to differential induction of vitellogenin protein in different tissues.

DISCUSSION

Vitellogenin influences hormone signalling, behavioural transition, stress resistance and longevity in honey bee workers (Amdam et al. 2004b, Nelson et al. 2007, Seehuus et al. 2006). Exposure of honey bees to PPPs including neonicotinoids, pyrethroids and organphosphates

altered the expression of vitellogenin on mRNA level in the brain (Christen et al. 2016; Christen and Fent 2017) but the induction on the protein level remained elusive. In our present work, we generated a polyclonal antibody against honey bee vitellogenin, confirmed it by GC-MS/MS determination, and analysed vitellogenin protein expression patterns in hemolymph, fat body, honey stomach and brain. We found cross-reactivity with three proteins that represent full-length and processed vitellogenin. We observed similar changes in vitellogenin protein levels in response to clothianidin as were reported previously for vitellogenin mRNA expression (Christen et al. 2016).

Vitellogenin in different tissues

Vitellogenins represent a multigene superfamily together with insect apolipophorins. Honey bee vitellogenin was described as a 180 kDa monomeric phospholipoglycoprotein (Wheeler and Kawooya 1990). In addition to the 180 kDa full size vitellogenin, which was detected in the hemolymph and in the fat body of workers, a 150 kDa fragment was found in the ovaries of queens (Seehuss et al. 2007), in the hypopharyngeal glands of workers (Amdam et al. 2003) and during vitellogenin purification from queen hemolymph (Wheeler and Kawooya 1990). Therefore, lower molecular weight products could represent a degradation product (Wheeler and Kawooya 1990) or processed vitellogenin (Havukainen et al. 2011). The fat body is the major source of vitellogenin, which is then secreted into the hemolymph (Raikhel and Dhadialla 1992; Nilsen et al. 2011). Vitellogenin from the fat body was proposed to be cleaved from the 180 kDa full length vitellogenin into a 150 kDa C-terminal fragment and a 40 kDa N-terminal fragment (Havukainen et al. 2011) in abdominal fat body tissue. In addition, in fresh, ion-exchange purified hemolymph only full length vitellogenin is detectable (Havukainen et al. 2011). Only with time, a 150 kDa degradation product is formed. In non-purified hemolymph, bands of 70-75 kDa occurred in Western Blots in addition to the full length vitellogenin (Havukainen et al. 2011). In addition to the full-length 180 kDa and the