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

smaller 150 kDa vitellogenin, a 200 kDa band was detected in fat body, hemolymph and brain lysates. This band represents an uncharacterised honey bee protein, which shows cross-reactivity with the vitellogenin antibodies.

In the fat body, hemolymph and in the brain of workers, we detected full length vitellogenin slightly below 180 kDa (Fig. 2). Corona et al. (2007) localized the vitellogenin mRNA not in the brain of bees themselves but in fat cells tightly bound to the brain. As these cells cannot be separated during dissection, our analysis covers the brain and these fat cells together. In addition to the full length vitellogenin, we also detected a band slightly below 150 kDa vitellogenin in the fat body and in the hemolymph (Fig. 2). According to Havukainen et al. (2011) this band likely represents the processed vitellogenin in the fat body and a degradation product in the hemolymph. The detected vitellogenin bands were both slightly below the published sizes of 180 and 150 kDa (Havukainen et al. 2011), potentially due to anomalous migration of the protein standard or samples as a result of incomplete denaturation or binding of detergent to the protein (Dolnik and Gurske 2011). In the brain, the lighter vitellogenin band is almost not detectable. As the fat body is the major source of vitellogenin synthesis and the hemolymph is the distributor of vitellogenin in the honey bee body, the occurrence of both vitellogenin forms in these tissues makes sense. In the brain and associated fat cells and in the hypopharyngal glands, the lighter vitellogenin form is only present in nurse bees, which are responsible for the larval feeding (Amdam et al., 2003b).

In addition to full length and processed vitellogenin, we detected a band at 70 kDa in the hemolymph (Fig. 2). Havukainen et al. (2011) used ion exchange purified hemolymph to detect different vitellogenin bands of 40, 150 and 180 kDa. However, in raw hemolymph extract a band around 70-75 kDa was detected, which is similar to the 70 kDa band detected in our present work (Fig. 2). This protein may represent a degradation product of the full length or 150 kDa vitellogenin. Both the full-length and the 150 kDa fragments are post-translationally modified by phosphorylation and glycosylation (Havukainen et al. 2011). The loss of