have been shown to rely on endosymbionts. *A. domesticus* has been shown not to contain intracellular symbionts[21]. Thus, the data presented here demonstrating 18:2(n−6) synthesis in axenic fat body tissues from *A. domesticus* clearly indicate that insect tissue produces 18:2. This conclusion for *A. domesticus* does not rule out the possible contribution of bacteriocytes in *P. americana*, but together with the inability of bacteria to synthesize 18:2 and the lack of any contaminating microorganisms in the incubations, the case is also strong for 18:2 synthesis by insect tissue in *P. americana*.

Extensive studies in plant tissue have shown that the enzyme responsible for the conversion of oleic acid to linoleic acid, the Δ^{12} desaturase, requires oleic acid esterified to phosphatidylcholine as a substrate[1]. In contrast, studies in the house cricket[22] and American cockroach (Borgeson, unpublished results) using microsomal preparations show that the substrate for the Δ^{12} desaturase is oleoyl-CoA. The results from the study reported here show that it is insect tissue that contains the novel Δ^{12} desaturase that converts oleoyl-CoA to linoleate.

These data show for the first time unambiguously that certain insect species can synthesize linoleic acid de novo from acetate, indicating that desaturation of oleic acid to linoleic acid occurs in these insects. This single step, not possible in any vertebrate, enables the insect to switch fatty acids from one family, (n−9), to another family, (n−6), thus forming de novo the precursor for the physiologically important eicosanoid, arachidonic acid (20:4(n−6)), which is in turn precursor to prostaglandins. Both the American cockroach and house cricket can form arachidonic acid de novo[23,24]. This step releases those insects with this capability from a nutritional dependency on plant-derived fatty acids.

1 This work was supported by the National Science Foundation under grant DCB-8914417. We would like to thank Coby Schal for his generous gift of American cockroaches and Tania Kellermeyer for her excellent technical assistance.
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0014-4754/91/030238-04$1.50 + 0.20/0

Changes in the protein kinase C activity or rat sternomastoid muscle during development and after denervation

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Received 16 July 1990; accepted 18 September 1990

Summary. The relationship between the activity of protein kinase C (PKC) and muscle innervation was explored in the rat sternomastoid muscle (SM) from day 18 of gestation (E18) to adult age. Between E18 and birth, PKC activity rose 5-fold, and during the day after birth, diminished to a level characteristic of the mature muscle. The rise chiefly occurred in the neural part of the muscle, in both the membrane and the cytosol fractions. Between E18 and day 5 after birth, the ratios of membrane to cytosol PKC activity rose from 0.5 to 10 and 3 respectively in the neural and aneural parts of the muscle. Denervation of adult SM reduced PKC activity by half in the membrane fraction of the neural part but did not significantly change it in the membrane or cytosol fractions of the aneural parts. These results suggest that innervation plays an important part in determining the level of PKC activity in muscle.

Key words. Protein kinase C; sternomastoid muscle; development; denervation.

Protein kinase C (PKC) plays a central role in signal transduction and is very important in neuronal transmission [1, 2], but its role in muscular tissue has not been extensively investigated. In studies of PKC during in vitro myogenesis, changes were reported in its activity and subcellular distribution during the differentiation of fetal myoblasts and satellite cells isolated from adult skeletal muscles [3–7]. In a previous investigation [8], we showed that changes in PKC activity during fetal and postnatal development are brought about by innervation. In the present study, we further analyzed the changes in muscle PKC activity induced by innervation using the rat sternomastoid muscle (SM). This muscle was selected because all its neuromuscular end-plates are located in the median part, which can easily be dissected and separated from the aneural parts. PKC activity and its subcellular distribution were analyzed in rat SM during fetal and post-natal development and in adult after denervation. We demonstrated that, as previously observed during fetal and post-natal development of limb muscles, growth and denervation also induced changes in PKC activity in the SM.

Materials and methods

White Wistar rats were anesthetized with ether. Sternomastoid muscles were excised from fetuses on day 18 of gestation (E18), from neonates on days 5 and 14 after birth and from adults at 2 months. The isolated muscles were rinsed with cold Ca^{2+} and Mg^{2+}-free PBS, and cut into 3 parts: the middle part containing the synaptic motor end-plate, known as the neural part, and the two extremities, devoid of synaptic motor end-plates, known as the aneural parts.

In another set of experiments, adult rats were anesthetized and the right sternomastoid motor nerve was cut close to its entrance into the muscle, dissected and implanted into other tissue to prevent spontaneous SM reinnervation, as previously described [9]. Three days after denervation, the rat was killed and the SM muscle was dissected as described above. The intact left SM muscle was taken as control.

Preparation of cellular fractions. Muscles were homogenized in a glass-glass conical homogenizer in buffer A containing 2 mM EDTA, 10 mM EGTA, 2 mM PMSF, 100 µg/ml leupeptine and 20 mM Tris-HCL pH 7.5. The crude extracts were centrifuged at 1000 × g for 10 min and the resulting supernatants were centrifuged for 1 h at 100,000 × g. The second supernatants constituted the cytosol fraction. The 100,000 × g pellets were left for 1 h at 4 °C in the buffer A to which 0.1% Triton X-100 had been added, and again centrifuged at 100,000 × g for 1 h. The resulting supernatants, referred to below as the membrane fractions were treated in the same way as the cytosol fractions.

Assay of PKC activity. PKC activity was assayed in either crude cytosol and membrane fractions, or in these fractions after their elution from DE-52 cellulose columns with a NaCl gradient from 0.0 to 0.3 M as previously described [10]. The crude membrane fractions were diluted before PKC assay so that the Triton X-100 concentration in the assay did not exceed 0.015%. PKC activity was measured by the incorporation of ^{32}P from ^{32}P-γ-ATP into histone type H III-S. The fractions (40 µg protein/ml) were incubated at 30 °C for 5 min with 0.2 mg/ml histone in 20 mM Tris-HCl pH 7.5, 5 mM EGTA, 5 mM $MgCl_2$, 10 µg/ml phosphatidylserine (PS) and 50 ng/ml 12-O-tetradecanoylphorbol 13-acetate (TPA). Radioactivity was determined with a liquid scintillation spectrometer by Cerenkov radiation. PKC activity was expressed as the difference between histone phosphorylation levels in the presence and absence of PS and TPA.

Assay of choline acetyltransferase (ChAT) activity. ChAT activity was determined by a method using [^{3}H]acetylcoenzyme A [11] and expressed as counts per minute of synthesized [^{3}H]acetylcholine.

Results

We first compared PKC activity and its subcellular distribution in the SM of fetal and neonate rats from day 18 of gestation to adult age. Important changes in this enzyme activity during growth had earlier been shown to occur in limb muscles [8]. Whatever the age of the animals, overall PKC activity was higher in the central neural part of the SM than in its aneural extremities (fig. 1 a, b). At E18, the activity in the cytosol fraction of the neural part was about 10 times higher than in the adult rat SM. At birth, overall PKC activity in the neural part, i.e. in both cytosol and membrane fractions, was 5–6 times the adult activity. In the cytosol fraction, PKC specific activity at birth reached 227 ± 16.4 pmol PO_4/mg prot/min in the neural part, and 42.1 ± 5.2 in the aneural part, compared to about half these levels in the membrane fractions (115.2 ± 5.8 and 26.3 ± 4.1, respectively). At day 5 after birth, PKC activity decreased about 5-fold in both the neural and aneural parts, but in the neural part it remained greater in the cytosol than in the membrane fraction. At day 14 after birth, the subcellular distribution of this activity changed as it rose to a higher level in the membrane than in the cytosol fraction in both the neural and aneural parts of the muscle (fig. 1). In the adult rat, most of the PKC activity in the SM was located in the membrane fractions, in both the neural and aneural parts, as we previously observed in adult hind limb muscles [8]. The ratios of membrane to cytosol activity which were between 0.5 and 0.6 at E18 and after birth rose to positive values, first in the aneural part by day 5, and then in the neural part by day 14 (fig. 2).

We also measured PKC activity in the cytosol and the membrane fractions of both the neural and aneural parts of the SM after their partial purification on DE-52 cellulose. The results confirmed that in both parts PKC activity reached its peak at birth (fig. 3).

Our observation showing that PKC activity was higher in the neural than in the aneural part of the muscle suggest-

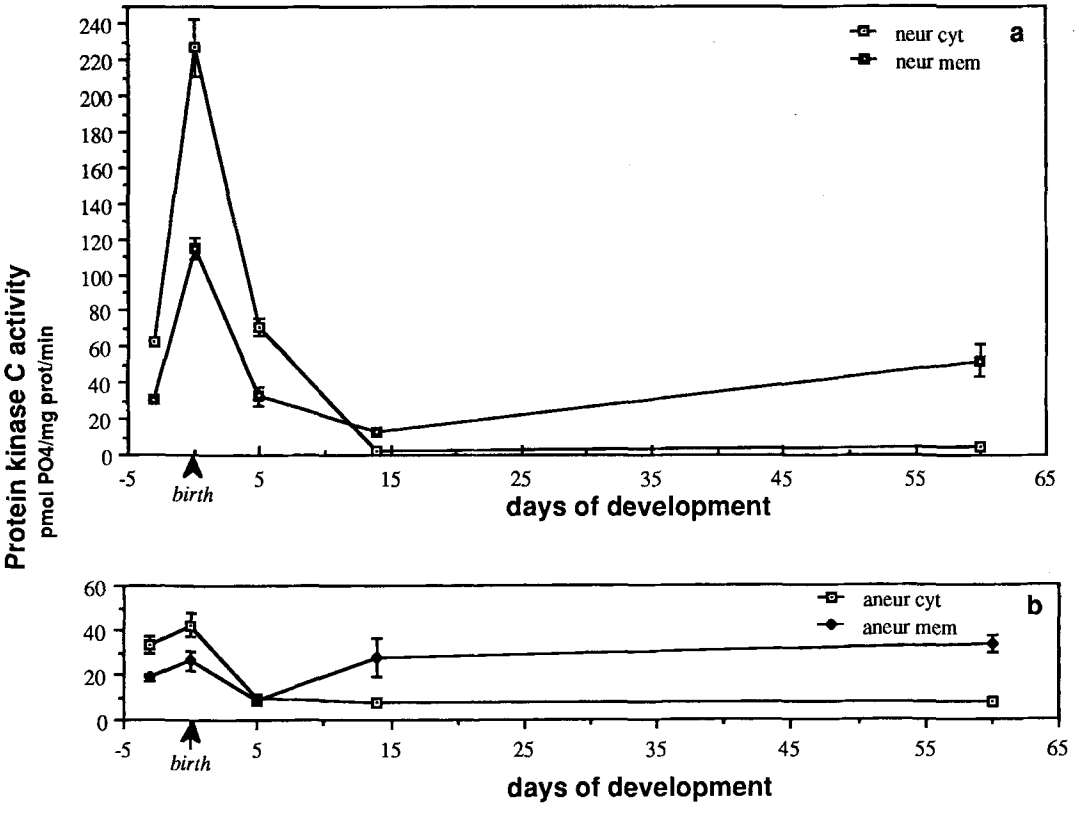

Figure 1. Variation in protein kinase C activity in crude cytosol and membrane fractions of neural (*a*) and aneural (*b*) parts of rat sternomastoid muscle during development and growth. Points are means ± SEM (n = 7).

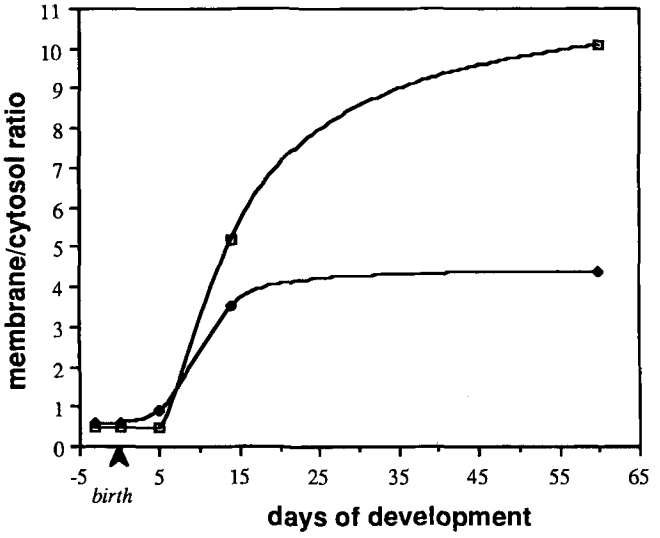

Figure 2. Ratios of membrane to cytosol protein kinase C activity in crude membrane and cytosol fractions of rat sternomastoid muscle during development and growth. The values are calculated from results of fig. 1. ⊡ neural part; ◆ aneural part.

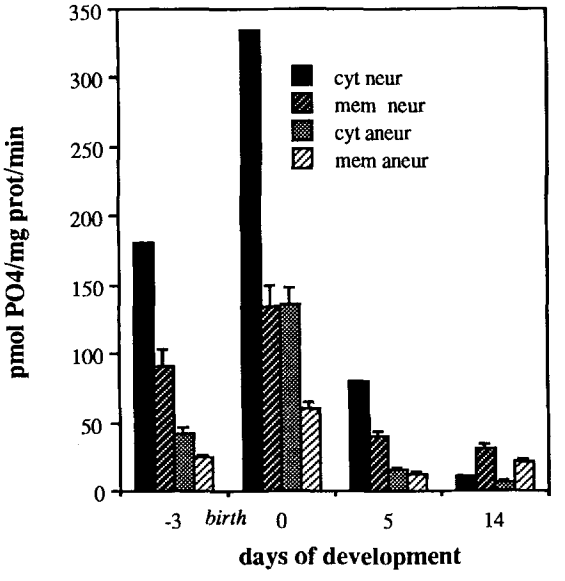

Figure 3. Variations in the activity of protein kinase C partially purified on a DE52 cellulose column in the neural and aneural parts of rat sternomastoid muscle during development and growth. Points are means ± SEM (n = 5).

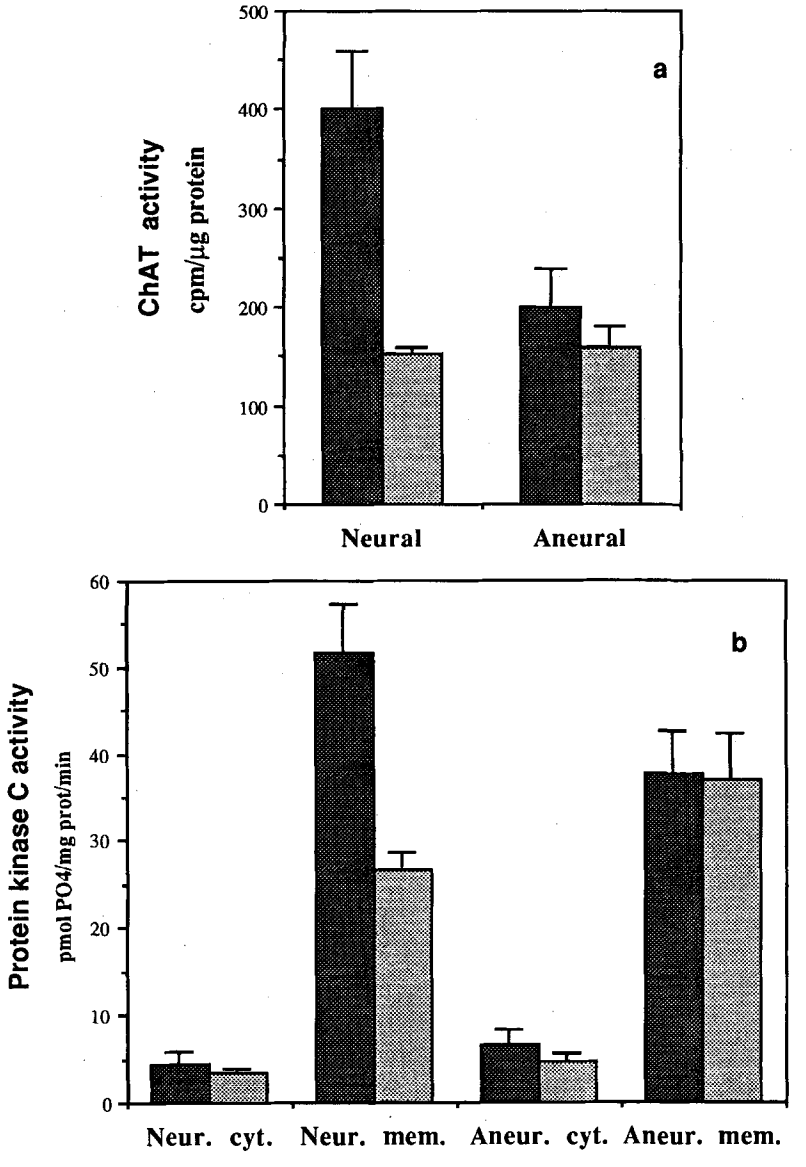

Figure 4. Effect of denervation on the activities of choline acetyltransferase (*a*) and protein kinase C (*b*) in neural and aneural parts of SM. Points are means ± SEM (n = 5). ▩ control SM; ▩ denervated SM.

ed that the presence of the nerve is important in determining this activity. To explore this hypothesis, we studied the effect of denervation of PKC activity in the membrane and cytosol fractions of the adult SM muscle. The effectiveness of denervation was checked by comparing the activity of choline acetyltransferase (ChAT) in the neural and aneural parts of denervated muscle and of intact muscle, because denervation is known to reduce ChAT activity in the neural part to its level in the aneural part[13]. Three days after denervation, ChAT activity in the neural part had dropped by 60% and was down to the same level as in the aneural part (fig. 4a). Overall PKC activity in the neural part of denervated muscle was about half that in the intact contralateral muscle (Fig. 4b). Most of this decrease occurred in the membrane fraction, and it was not significant in the cytosol fraction. In contrast, in the aneural parts of the SM, denervation did not significantly alter PKC activity or its subcellular distribution.

Discussion

The main purpose of this work was to establish a relationship between PKC activity and innervation in rat SM. In the adults, the structure and innervation of adult rat SM are well known[12], and the changes induced by denervation in acetylcholinesterase activity have been described in the neonate and the adult rats[13]. However, nothing was known about the pattern of SM innervation during development, and we therefore consider the present results in the light of our previous observation that

during fetal and post-natal development, PKC activity in hind limb muscles is determined by innervation. In these muscles, we previously showed[8] that this activity increased during two crucial periods of muscle development – the polyinnervation phase (E18), and at day 5 after birth, when regression of polyinnervation takes place. In the present study, we observed that during the same periods, PKC activities in SM only displayed one peak, at birth, in both the cytosol and membrane fractions. By E18, it was already higher in SM than in limb muscles, and increased until birth instead of decreasing. We concluded that the level of PKC activity in SM is determined by changes in muscle innervation as in limb muscles. This was supported by the results obtained after SM denervation. The drastic decrease in the PKC activity of the membrane fraction of the neural part of denervated SM muscle showed that most of this activity was due to the presence of the nerve ending. Thus, the discrepancy we observed between the patterns of PKC activity in growing SM and limb muscles might be attributable to differences between these muscles with regard to the times at which innervation is acquired and stabilized during development. Innervation might mature earlier in the SM, which is involved in suckling, than in limb muscles. The shift of most of the PKC activity from the cytosol to the membrane fractions observed in 14-day-old and adult SM can be interpreted as follows. In tissue in which PKC is physiologically active its activity is mostly membranous[15]. Enzyme translocation from the cytosol to the membrane fraction is considered to be PKC activation[16]. Thus, in our experiments, stronger PKC activity in the membranous fraction of 14-day-old and adult SM might signify permanently stabilized muscle innervation. The high level of PKC activity observed during the neonate period, i.e. during intensive muscular differentiation and polyinnervation, probably corresponded, on the contrary, to a phase of intense synthesis of PKC which then accumulated principally in the cytosol.

Acknowledgments. This work was supported in part by PAN (grant CPBP 04-12), by INSERM (grant 876015) and by ATP Franco-Polonaise (n° 2C15).

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0014-4754/91/030241-05$1.50 + 0.20/0

Subtypes of protein kinase C in rat cerebral microvessels

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Received 28 May 1990; accepted 3 September 1990

Summary. Protein kinase C in rat cerebral microvessels was characterized. By hydroxyapatite column chromatography, protein kinase C in the soluble fraction was resolved into two major peaks corresponding to type II and III enzymes, in the proportions of 57% and 38%, respectively. Since each subtype is considered to have a distinct role, the high proportion of type II enzyme found in this study suggests that this type may be involved in specific functions of the cerebral microvessels.
Key words. Cerebral microvessel; protein kinase C; subtype.

Elucidation of the mechanisms that regulate the physiological function of the cerebral microvessels is important for a better understanding of cerebral disorders, and may give us a clue to methods for their clinical treatment. Several biological phenomena occur through protein phosphorylation and dephosphorylation. The presence