SBD, a novel structural subunit of the *Drosophila* nicotinic acetylcholine receptor, shares its genomic localization with two α-subunits

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Nicotinic acetylcholine receptors (nAChRs) display marked heterogeneity in both vertebrates and invertebrates. Here we describe the structure of a cDNA from *Drosophila melanogaster* which encodes a novel nAChR β-type subunit (SBD or β2). The deduced amino acid sequence of SBD displays remarkable similarity to the *Drosophila* α-subunits, ALS and SAD, while homology to the *Drosophila* β-subunit ARD is less pronounced. The temporal expression of *sbd* transcripts during *Drosophila* development is similar to that of other nAChR subunit mRNAs, with high levels being found during late embryonic and late pupal stages. In embryos, *sbd* and *als* transcripts are localized in the central nervous system. The *sbd* gene maps cytogenetically in proximity to the *als* and *sad* genes at position 96A of chromosome 3R, suggesting the existence of a nAChR gene cluster in invertebrates.

Nicotinic acetylcholine receptor; Nervous system; Receptor heterogeneity; Gene cluster; *Drosophila melanogaster*

1. INTRODUCTION

The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel which mediates synaptic excitation of both muscle and nerve cells. Biochemical and molecular cloning studies indicate that this receptor is composed of homologous membrane spanning subunits that share significant sequence homology and a common transmembrane topology with subunits of other ion channel forming receptors [1–3]. cDNA sequencing and heterologous expression of several nAChR subunits indicate that cholinergic signal transmission in the central nervous system (CNS) involves different nAChR subtypes of distinct pharmacological and molecular properties. Besides the five different subunits (α,β, γ, δ, ε) of the neuromuscular nAChR [1], four additional α or ligand binding subunits (α2–α5) and three β or structural subunits (β2–β4) of neuronal origin have been characterized in vertebrates [1,4–10]. Furthermore, the primary structures of two neuronal α-like proteins pharmacologically related to the muscular nAChR have been described recently [11]. Common structural features and significant sequence homology are shared by all these nAChR proteins, suggesting that they evolved from a common ancestor by gene duplication.

In invertebrates, heterogeneity of neuronal nAChRs has also been disclosed by molecular cloning. For *Drosophila melanogaster*, genomic and cDNAs encoding one β-type (ARD) [12,13] and two α-like (ALS and SAD) [14,15] neuronal nAChR proteins have been isolated. We have recently shown that at least two further nAChR related genes must exist in this organism [15]. Here we report the isolation of cDNAs encoding a novel β subunit (SBD; second beta-like subunit of *Drosophila* or β2) that is developmentally regulated and shows remarkable similarity to the α-like polypeptides ALS and SAD. The gene encoding this polypeptide, the *sbd* locus, maps to the same region of *Drosophila* polytene chromosome 3R as *als* and *sad*, suggesting that at least three *Drosophila* nAChR genes exist in a gene cluster.

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2. MATERIALS AND METHODS

2.1. *Isolation of cDNA clones*

SBD-specific cDNA clones were isolated previously by homology screening using a genomic nAChR DNA fragment as probe [15]. Two positive clones (p2/1 and p6/1) were found to contain an overlapping open reading frame and are described here in detail.

2.2. *Polymerase chain reaction (PCR)*

PCR of cDNA synthesized from embryonic and head poly(A)$^+$ RNA was carried out using the Gene Amp kit according to the manufacturer's protocol (Perkin Elmer Cetus, Überlingen, FRG). Oligonucleotides derived from the SBD cDNA sequence, designated 1/13 (AAGCTGATGATGATGCG) and 1/9 (AGGAATCTCGCTCAGCT) (see Fig. 1a) were used as primers. PCR was performed for 30 cycles (denaturation for 20 s at 94°C, annealing for 30 s at 55°C, and elongation for 1 min at 72°C) with a programmable thermocycler. PCR-amplified DNA was separated in 1.5% agarose gels and visualized after staining with ethidium bromide.

2.3. *RNA isolation and Northern blot analysis*

Isolation of poly(A)$^+$ RNA and Northern blot hybridization were

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carried out as described [15]. The ^{32}P-labelled *Bgl*II-*Eco*RI fragment (nucleotides 947–1711) of clone p6/1 was used as hybridization probe.

2.4. *Whole mount in situ hybridization*

Localization of *sbd* transcripts in *Drosophila* embryos was performed as described [15,16]. The digoxigenin-labelled clone p6/1 was used as hybridization probe. *als* transcripts were visualized using the *Bst*EII-*Eco*RI fragment of the ALS cDNA which encompasses the cytoplasmic region, transmembrane segment M4, the C-terminus and a part of the 3′-untranslated region [14].

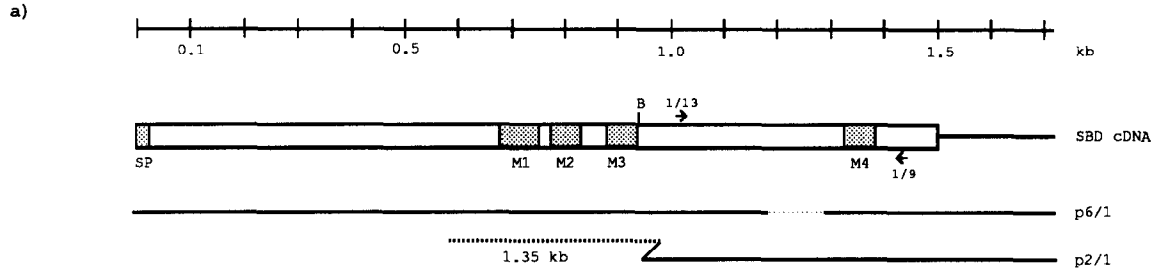
2.5. *In situ hybridization to polytene chromosomes*

Chromosomal in situ hybridizations were performed essentially as described in [17]; however, digoxigenin-labelled probes were used. For labelling of probes and detection of hybridization signals, a nonradioactive labelling kit was used according to the manufacturer's protocol (Boehringer, Mannheim, FRG). Hybridization of labelled p6/1 SBD cDNA and p37 SAD cDNA [15] to *Drosophila* polytene chromosomes was done at 63°C in 5×SET (750 mM NaCl, 5 mM EDTA, 150 mM Tris/HCl, pH 8.0), 0.1% (w/v) SDS, 0.1% (w/v) sodium pyrophosphate, 10× Denhardt's solution and 200 μg/ml denatured herring sperm DNA. Probing of control Southern blots under similar conditions to those employed for chromosomal localization showed no cross-hybridization between the different cDNA probes used.

3. RESULTS

3.1. *Isolation and characterization of SBD cDNA clones*

SBD encoding cDNA clones were isolated from an embryonic *Drosophila* λ-Zap cDNA library by screening with genomic DNA fragments originally selected by hybridization to a consensus nAChR subunit sequence [15]. The cDNA sequence covered by the clones p2/1 and p6/1 consists of 1714 nucleotides (Fig. 1). The region 5′ of position 949 in clone p2/1 corresponds to intronic sequence. Nucleotides 1182–1288 coding for a putative amphiphilic α-helix [1] were not found in clone p6/1. Polymerase chain reaction (PCR) with an appropriate oligonucleotide combination (see Fig.1a) on



b)

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   1  GGCTCCAATAAGTTTTGAGGCAAATCCGGACACCAAACGTCTCTATGATGATTTGTTGAGCAACTACAATCGATTGATTAGGCCCGTTGTGAACAACACGGAGACCCTGACGGTTTGGTT
  -7  - A P I S F E A N P D T K R L Y D D L L S N Y N R L I R P V V [N] N T E T L T V W L

 121  AGGTCTGAAATTGTCTCAGCTGATTGAGGTCAATCTGAAGAATCAGGTGATGACCACCAATCTGTGGGTGAAGCAGCGCTGGTTCGACTACAAGTTGCGATGGGATCCAGAGGAATACGG
  34    G L K L S Q L I E V N L K N Q V M T T N L W V K Q R W F D Y K L R W D P E E Y G

 241  CGGAGTTGAACAGCTGTATGTGCCATCTGAGCACATTTGGGTACCGGACATTGTGTTGTACAACAACTGGGATGGCAACTATGAAGTAACTCTGATGACGAAAGCCACTCTGAAGTATAC
  74    G V E Q L Y V P S E H I W V P D I V L Y N N W D G N Y E V T L M T K A T L K Y T

 361  GGGAGAAGTCTTTTGGGAGCCACCTGCTATATACAAGTCATCCTGTGAGATGAATGTCGAATACTTTCCGTACGACGAACAAATTTGCTTCATGAAATTCGGTTCATGGACCTACAATGG
 114    G E V F W E P P A I Y K S S © E M N V E Y F P Y D E Q I © F M K F G S W T Y N G

 481  TGCCCAAGTGGATCTGAAGCATTTGGATCAGATTCCTGGCAGTAACCTAGTGCAAGTTGGCATCGATCTCACGGAATTTTATTTGTCTGTGGAATGGGACATACTTGAAGTGCCGGCTAC
 154    A Q V D L K H L D Q I P G S N L V Q V G I D L T E F Y L S V E W D I L E V P A T

 601  CAAAAATGAGGAGTATTACCCCGATACACTGGAACCCTTTTCAGATATCACATTTAAGTTGACCATGCGGCGTAAGACCTTGTTCTATACCGTTAATTTGATTGTGCCATGTGTGGCTTT
 194    K N E E Y Y P D T L E P F S D I T F K L T M R R K T L F Y T V N L I V P C V A L

 721  AACATTCCTCACGGTGTTGGTCTTTTATTTGCCCAGCGATTCGGGCGAAAAGGTTACGTTATGTATTTCGATACTCGTGTCGTTGACCGTGTTTTTCCTGCTCTTGGCCGAAATTATCCC
 234    T F L T V L V F Y L P S D S G E K V T L C I S I L V S L T V F F L L L A E I I P

 841  CCCAACCTCGTTGGCCGTGCCCCTGCTGGGCAAGTACCTTCTCTTTACCATGATACTCGTCTCGCTATCCGTTTGGACGACGGTCTGCGTGCTGAACATCCATTTCAGATCTCCATCTAC
 274    P T S L A V P L L G K Y L L F T M I L V S L S V W T T V C V L N I H F R S P S T

 961  GCACAATATGTCGCGGCTGGTGCGGAAACTCTTCCTGCACTTCATGCCCAAGCTGATGATGATGCGGCGGACGCAGTACACTCTGCCCGACTACGATGACTCCACTCCGAGCAATGGGTA
 314    H N M S R L V R K L F L H F M P K L M M M R R T Q Y T L P D Y D D S T P S N G Y

1081  CACCAACGAGATCGATGTGCGTGACAGCATCAGCGATTTTCCCAGCGAGTTTAAGGACAGCCAGGATGGCGCTTACGATAATGGCATGCAGAACTCTGTGGATTCCGACAATGTGATACC
 354    T N E I D V R D S I S D F P S E F K D S Q D G A [Y] D N G M Q N S V D S D N V I P

1201  GCGCAACTTAACACCCGAAGTGCTGCAGGCGTTACGTGCGGTCAGATTTATTGCGCAGCATATCAAGGATGCCGACAAGGATAACGAGATTGTGGAGGACTGGAAGTTCGTATCGATGGT
 394    R N L T P E V L Q A L R A V R F I A Q H I K D A D K D N E I V E D W K F V S M V

1321  GCTGGACCGCTTCTTTCTCTGGCTCTTCACGCTGTCCTGTGTGTTCGGGACCTTGGCCATAATATGTCAGTCGCCATCGCTATATGACACCCGGTCGCCCATTGACCGCCAGCTGAGCGA
 434    L D R F F L W L F T L S C V F G T L A I I C Q S P S L Y D T R S P I D R Q L S E

1441  GATTCCTTTGCGCAAAAACAATTTCATGCTGCCGCCGGACATTGTCCGTCAGGTCCTAACCTAATTGGGGTCCCGAGGTCTTCCCGTCTGAGTTAACAATGCCTCCGAGGCTCGGAACTG
 474    I P L R K N N F M L P P D I V R Q V L T *

1561  TGTTTTTTATGATGCGCAATCAAATCAGTTGCTTGCTGGGAATGCGGCAACGATTTATGTCTAAGTGTTTATCATCCTGCAATGCTGTAAAACCGAATTAGCTATAAAACGTGGAAACTA
1681  AATAAAAAAAATTCTGTTTTAACTCATAACTTCA
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Fig. 1(a) Map of SBD cDNA clones p2/1 and p6/1. The protein coding region is indicated by an open box. Putative transmembrane segments (M1–M4), part of the signal peptide (SP), and the recognition site for *Bgl*II (B) are indicated. Arrows show the location of oligonucleotides 1/9 and 1/13 used for PCR. A dotted line indicates nucleotide sequence not contained in clone p6/1, and a stippled one intronic sequence in clone p2/1. (b) Nucleotide and deduced amino acid sequence of overlapping SBD cDNA clones. Part of the putative signal peptide, the membrane spanning segments and a potential polyadenylation signal are underlined. The N-linked glycosylation site and a potential tyrosine kinase phosphorylation site are boxed, and the conserved cysteine residues are marked by circles.

cDNA derived from poly(A)$^+$ RNA isolated from *Drosophila* embryos and adult heads revealed a major amplification product corresponding in length to the sequence contained in clone p2/1 (data not shown). Both clones also differ slightly at their 3′ ends where clone p6/1 lacks the last 3 nucleotides. Both cDNAs define an open reading frame that extends between nucleotides 1–1501 followed by 3′-untranslated sequence harbouring a polyadenylation signal at position 1681.

3.2. *The deduced SBD protein displays high homology to* Drosophila *α-subunits*

The mature SBD protein predicted from clones p2/1 and p6/1 consists of 493 amino acid residues with a calculated molecular mass of 57 270. All conserved structural elements of nAChR polypeptides including a signal peptide (not complete in clone p6/1), four putative membrane spanning regions and a conserved domain with two cysteine residues spaced 13 amino acids apart are found in the deduced SBD protein. One possible N-linked glycosylation site is located near the N-terminus at amino acid position 24 and a potential phosphorylation site for tyrosine kinases in the cytoplasmic region is situated at position 378. Since the SBD protein does not contain the two tandem cysteine residues common to ligand binding nAChR α-subunits [1], we classify this protein as a β-type subunit. As in other members of the nAChR family, a putative amphipathic helix is found in front of the fourth transmembrane segment. An alignment of the SBD protein with known nAChR subunits of *Drosophila* is shown in Fig. 2. High homology is seen with the α-like subunits ALS (α1) and SAD (α2), whereas similarity to the ARD (β1) protein is less pronounced. Calculated amino acid identities of SBD to ALS, SAD, and ARD polypeptides are 56%, 54% and 41%, respectively.

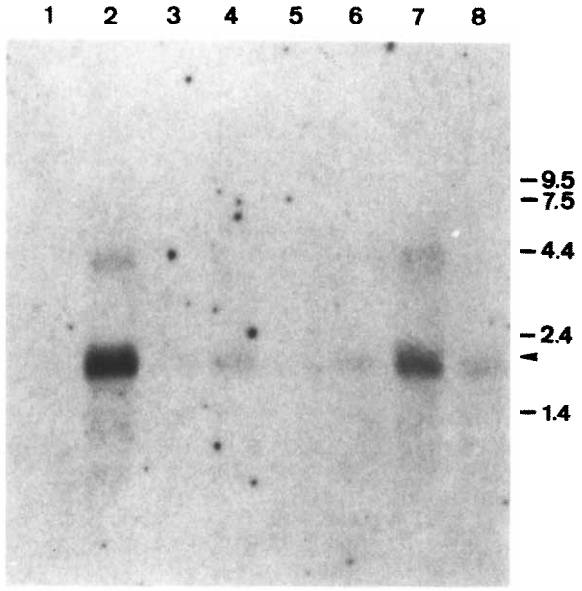

Fig. 3. Nothern blot analysis of poly(A)$^+$ RNA (5 μg/lane), isolated at different stages of *Drosophila* development: (1) early embryos (0–4 h); (2) late embryos (14–22 h); (3) first instar larvae; (4) second instar larvae; (5) third instar larvae; (6) early pupae; (7) late pupae; (8) early adults (1–2 days). Sizes of RNA length standards are given in kb. The *Bgl*II-*Eco*RI fragment of clone p6/1 was used as ^{32}P-labelled hybridization probe.

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   +++ +*******+++**+***** **+      *  ** ++***  *++ *  +++ *     * *    * +  ***+  * +*+++++* *******+**
 1 NPDTKRLYDDLLSNYNRLIRPVVNNTETLTVWLGLKLSQLIEVNLKNQVMTTNLWVKQRWFDYKLRWDPEEYGGVEQLYVPSEHIWVPDIVLYNN SBD (β2)
 1 NPDAKRLYDDLLSNYNRLIRPVGNNSDRLTVKMGLRLSQLIDVNLKNQIMTTNVWVEQEWNDYKLKWNPDDYGGVDTLHVPSEHIWHPDIVLYNN ALS (α1)
 1 NPDAKRLYDDLLSNYNRLIRPVSNNTDTVLVKLGLRLSQLIDLNLKDQILTTNVWLEHEWQDHKFKWDPSEYGGVTELYVPSEHIWLPDIVLYNN SAD (α2)
 1 SEDEERLVRDLFRGYNKLIRPVQNMTQKVGVRFGLAFVQLINVNEKNQVMKSNVWLRLVWYDYQLQWDEADYGGIGVLRLPPDKVWKPDIVLFNN ARD (β1)

    ** * *+ ++++ +  ** * * **** +* *+  * *** * * *+******+ *   +* +  +     +    ++**   + *++***
96 WDGNYEVTLMTKATLKYTGEVFWEPPAIYKSSCEMNVEYFPYDEQICFMKFGSWTYNGAQVDLKHLDQIPGS NLVQVGIDLTEFYLSVEWDILE SBD (β2)
96 ADGNYEVTIMTKAILHHTGKVVWKPPAIYKSFCEIDVEYFPFDEQTCFMKFGSWTYDGYMVDLRHLKQTADSDN IEVGIDLQDYYISVEWDIMR ALS (α1)
96 ADGEYVVTTMTKAILHYTGKVVWTPPAIFKSSCEIDVRYFPFDQQTCFMKFGSWTYDGDQIDLKHISQKNDKDNKVEIGIDLREYYPSVEWDILG SAD (α2)
96 ADGNYEVRYKSNVLIYPTGEVLWVPPAIYQSSCTIDVTYFPFDQQTCIMKFGSWTFNGDQVSLALYNNKNF         VDLSDYWKSGTWDIIE ARD (β1)

   ***   +  +    +*   ** *  + ************ *+*    * ******* ++**** *+***** * + *+**  +*+* *** **++
190 VPATKNEEYYPDTLEPF SDITFKLTMRRKTLFYTVNLIVPCVALTFLTVLVFYLPSDSGEKVTLCISILVSLTVFFLLLAEIIPPTSLAVPLLG SBD (β2)
190 VPAVRNEKFYSCCEEPY LDIVFNLTLRRKTLFYTVNLIIPCVGISFLSVLVFYLPSDSGEKISLCISILLSLTVFFLLLAEIIPPTSLTVPLLG ALS (α1)
191 VPAERHEKYYPCCAEPY PDIFFNITLRRKTLFYTVNLIIPCVGISYLSVLVFYLPADSGEKIALCISILLSQTMFFLLISEIIPSTSLALPLLG SAD (α2)
183 VPAYLNV YEGDSNHPTETDITFYIIIRRKTLFYTVNLILPTVLISFLCVLVFYLPAEAGEKVTLGISILLSLVVFLLLVSKILPPTSLVLPLIA ARD (β1)
                                  M1                                                  M2
   *******+ ++ +*+  *   +*   * * ** *       *    *++
284 KYLLFTMILVSLSVWTTVCVLNIHFRSPSTHNMSRLVRKLFLHFMPKLMMMRRTQYTLPDYDDSTPSNGYTNEIDVRDSISDFPSEFKDSQDGAY SBD (β2)
284 KYLLFTMMLVTLSVVVTIAVLNVNFRSPVTHRMAPWVQRLFIQILPKLLCIERPKKEEPEEDQPPEVLTDVYHLPPDVDKFVNYDSKRFSGDYGI ALS (α1)
285 KYLLFTMLLVGLSVVITIIILNIHYRKPSTHKMRPWIRSFFIKRLPKLLLMRVPKDLLRDLAANKINYGLKFSKTKFGQALMDEMQMNSGGSSPD SAD (α2)
277 KYLLFTFIMNTVSILVTVIIINWNFRGPRTHRMPMYIRSIFLHYLPAFLFMKRPRKTRLRWMMEMPGMSMPAHPHPSYGSPAELPKHISAIGGKQ ARD (β1)
              M3
                                                                          *          **  *
379 DNGMQNSVDSDNVIP                                                  RNLTPEVLQALRAVRFIAQHIKDA SBD (β2)
379 PALPASHRFDLAAAGGISAHCFAEPPLPSSLPLPGADDDLFSPSGLNGDISPGCCPAAAAAAAADLSPTFEKPYAREMEKTIEGSRFIAQHVKNK ALS (α1)
380 SLRRMQGRVGAGGCNGMHVTTATNRFSGLVGALGGGLSTLSGYNGLPSVLSGLDDSLSDVAAR        KKYPFELEKAIHNVMFIQHHMQRQ SAD (α2)
372 SKMEVMELSDLHHPNCKINRKVNSGGELGLGDGCRRESESSDS                          ILLSPEASKATEAVEFIAEHLRNE ARD (β1)

    *       **  * ** ** +*+ *       **  *    *++ +    ++   +
418 DKDNEIVEDWKFVSMVLDRFFLWLFTLSCVFGTLAIICQSPSLYDTRSPIDRQLSEIPLRKNNF                              SBD (β2)
474 DKFESVEEDWKYVAMVLDRMFLWIFAIACVVGTALIILQAPSLHDQSQPIDILYSKIAKKKFELLKMGSENTL                    ALS (α1)
467 DEFNAEDQDWGFVAMVMDRLFLWLFMIASLVGTFVILGEAPSLYDDTKAIDVQLSDVAKQIYNLTEKKN                       SAD (α2)
439 DLYIQTREDWKYVAMVIDRLQLYIFFIVTTAGTVGILMDAPHIFEYVDQDRIIEIYRGK                                  ARD (β1)
                       M4
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Fig. 2. Alignment of the deduced mature SBD protein with *Drosophila* nicotinic acetylcholine receptor subunits ALS [14], SAD [15] and ARD [12,13]. Stars indicate positions conserved in all subunits. Amino acid residues identical in SBD, ALS and SAD are marked by crosses. Transmembrane regions M1–M4 are underlined.

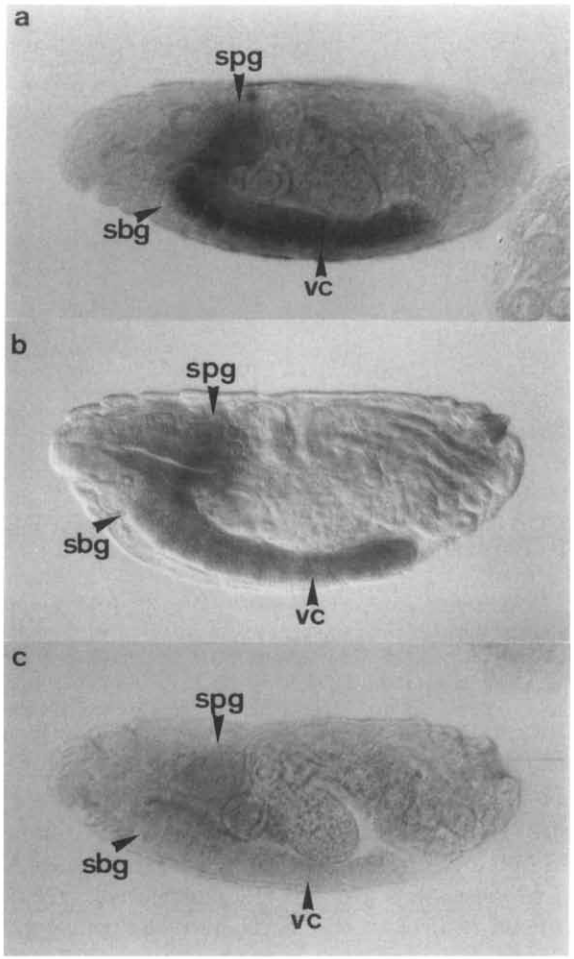

Fig. 4. Local distribution of *sbd* and *als* transcripts in *Drosophila* late embryos. Whole mount in situ hybridization was carried out using the digoxigenin-labelled SBD clone p6/1 (a) and the ALS cDNA fragment *Bst*EII-*Eco*RI [14] (b) as probes. Control hybridization (c) was performed with digoxigenin-labelled phagemid DNA (Bluescript SK). Arrowheads indicate: spg, supraoesophageal ganglia; sbg, suboesophageal ganglia; vc, ventral cord.

3.3. *Developmental accumulation and localization of* sbd *transcripts*

Expression of the *sbd* gene during *Drosophila* development was investigated by Northern blot analysis (Fig. 3). In early embryos the *sbd* gene is not expressed, whereas in late embryos high levels of a 2.0 kb *sbd* transcript were found. At larval stages, detectable amounts of *sbd* mRNA were seen only in second instar larvae. Expression of the *sbd* gene was also low during early pupation, but high amounts of corresponding transcripts appeared in late pupae. In early adult flies, again a decreased level of expression was observed.

In situ hybridization with digoxigenin-labelled probes to whole mount embryo preparations were performed to determine the tissue distribution of *sbd* transcripts. During late embryogenesis, intense staining with an SBD probe was observed in the sub- and supraoesophageal ganglia and the ventral cord (Fig. 4a). Hybridization with an ALS-specific probe also resulted in staining of the same parts of the embryonic CNS (Fig. 4b). As shown in Fig. 4c, no staining of neuronal tissue was observed when digoxigenin-labelled plasmid DNA was used as hybridization probe. We therefore conclude that, in embryos, *sbd* transcripts are exclusively localized in the nervous system.

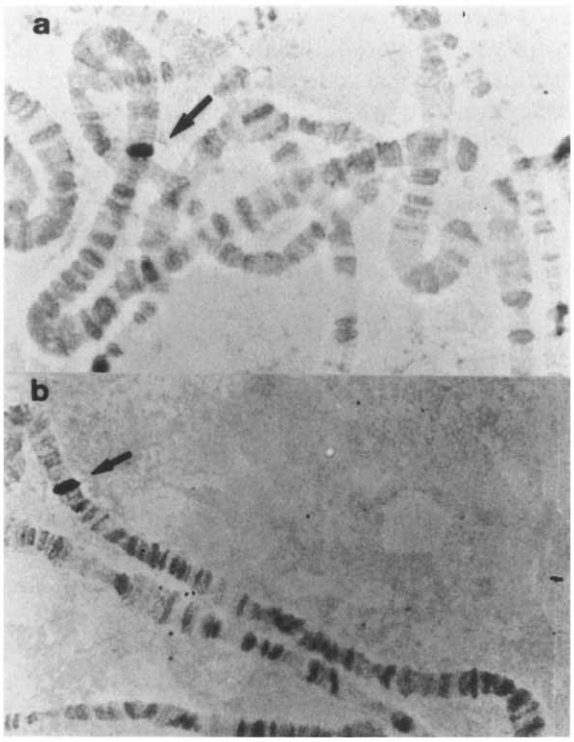

Fig. 5. In situ hybridization to polytene chromosomes. The digoxigenin-labelled SBD clone p6/1 (a) and SAD clone p37 [15] (b) both hybridize to the 96A region of chromosome 3R (arrows).

3.4. *The* sbd *gene maps cytogenetically close to the* als *and* sad *genes*

In situ hybridization with both SBD and SAD cDNA probes to polytene chromosomes revealed intense staining of region 96A of the third chromosome (Fig. 5). This corresponds to the position of the *als* gene [14] and indicates that at least three *Drosophila* nAChR genes may be arranged as a gene cluster.

4. DISCUSSION

Previously, three homologous nAChR subunits have been identified in *Drosophila*, the α-like proteins ALS or $\alpha 1$ [14], SAD or $\alpha 2$ [15] and the β-subunit ARD (which now may be classified as $\beta 1$) [12,13]. Here, we have obtained the sequence of a novel nAChR β-type subunit (SBD or $\beta 2$). This polypeptide shows remarkable similarity to the *Drosophila* neuronal α-subunits. As already described for the other *Drosophila* nAChR subunits [12–15], homology of the SBD protein is higher to vertebrate neuronal than to vertebrate muscular nAChR polypeptides.

Comparison of the expression of the *sbd* gene during *Drosophila* development with that of other nAChR genes shows similar temporal patterns of mRNA accumulation [12,13,15,18]. High transcript levels are observed in late embryos and late pupae, i.e. during the major periods of neurogenesis in the fruitfly. An exception is the *als* gene, where high levels of mRNA are also seen in second instar larvae [14], while *sbd* gene expression during this period is significantly decreased. Also, *sad* and *ard* genes seem not to be expressed during this stage.

As previously shown for *ard* [13,18] and *sad* [15], expression of *sbd* and *als* genes during late embryogenesis is restricted to the CNS. This finding is consistent with earlier data where cholinergic synapses and binding sites for the snake venom component α-bungarotoxin (α-Btx), a specific blocker of the neuromuscular nAChR, have been shown to be abundant in the insect CNS [19–21].

The cytogenetic location of the *sbd* gene at position 96A of chromosome 3R is shared with both the *sad* and *als* genes, suggesting that these genes may be derived from a common ancestor by gene duplication. This assumption is supported further by the remarkable similarity of the deduced proteins which display extended stretches of identical amino acids in all 3 polypeptides. The *ard* gene in contrast maps to a different region (64B) of the third chromosome [13,17], and the corresponding protein shows a higher divergence from the other three subunits. In vertebrates, nAChR subunit gene clusters have been described for the muscular γ and δ [22–24] and for the neuronal α3, α5 and β4 subunit genes [10]. Thus, the two α and the β nAChR subunit genes localized at region 96A most likely represent a similar gene cluster in *Drosophila*. However, a more detailed analysis of this genomic region will be required to corroborate this conclusion.

The identification of the SBD cDNA shows that in *Drosophila* heterogeneity exists for both α-type and β-type nAChR subunits. A cDNA encoding a further α-like subunit is currently being analyzed in our laboratory. Thus at least 5 different nAChR proteins exist in the fruitfly. Heterogeneity of nAChRs in *Drosophila* is also indicated by pharmacological and immunological studies. At least two different high affinity binding sites for α-Btx are found in *Drosophila* head membrane preparations [25]. Recent immunoprecipitation experiments have shown that the ARD and ALS proteins both are components of an α-Btx binding complex (Schloss, P., Betz, H., Schröder, C. and Gundelfinger, E.D., submitted). Furthermore, different monoclonal antibodies against *Torpedo* nAChR displayed crossreactivity with distinct regional subsets of neural tissue in *Drosophila* [26]. Additional pharmacological, immunological and electrophysiological experiments will now be required to elucidate the complexity and subunit composition of nAChRs in *Drosophila*.

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