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# A novel soluble protein factor with non-opioid dynorphin A-binding activity

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**Abstract**

A novel soluble non-opioid dynorphin A-binding factor (DABF) was identified and characterized in neuronal cell lines, rat spinal cord, and brain. DABF binds dynorphin A(1–17), dynorphin A(2–17), and the 32 amino acid prodynorphin fragment big dynorphin consisting of dynorphin A and B, but not other opioid and non-opioid peptides, opiates, and benzomorphans. The $IC_{50}$ for dynorphin A(1–17), dynorphin A(2–17), and big dynorphin is in the 5–10 nM range. Using dynorphin A and big dynorphin fragments a binding epitope was mapped to dynorphin A(6–13). DABF has a molecular mass of about 70 kDa. SH-groups are apparently involved in the binding of dynorphin A since *p*-hydroxy-mercuribenzoic acid inhibited this process. Upon interaction with DABF dynorphin A was converted into Leu-enkephalin, which remained bound to the protein. These data suggest that DABF functions as an oligopeptidase that forms stable and specific complexes with dynorphin A. The presence of DABF in brain structures and other tissues with low level of prodynorphin expression suggests that DABF as an oligopeptidase may degrade other peptides. Dynorphin A at the sites of its release in the CNS may attenuate this degradation as a competitor when it specifically binds to the enzyme.


*Keywords:* Dynorphin; Binding site; Non-opioid effects; Oligopeptidase; Conversion

Dynorphin A, dynorphin B, and α-neoendorphin are the endogenous ligands for κ-opioid receptors. Several effects of dynorphin A, however, are not blocked by the general opioid antagonist naloxone and the κ-receptor selective antagonist nor-binaltorphimine [1–17]. The N-terminally truncated dynorphin A(2–17) that does not interact with opioid receptors is able to induce non-opioid effects characteristic of dynorphin A(1–17). Non-opioid effects of dynorphin A appear to be relevant for several pathophysiological processes including chronic neuropathic pain and spinal cord injury. The levels of dynorphin A are elevated in the spinal cord under conditions of chronic neuropathic pain and at the sites of spinal cord injury [1–4]. Dynorphin A produces neurological dysfunctions and hindlimb paralysis, and also a long-lasting state of allodynia through non-opioid mechanisms in rats [5–11]. Endogenous dynorphins are critically involved in the development of hyperalgesia and allodynia secondary

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to nerve injury or chronic opioid exposure, and their activities are not mediated via opioid receptors [8,9].

The glutamate receptors have been proposed to be involved in non-opioid effects of dynorphin A since NMDA receptor antagonists protect against hindlimb paralysis [10–15], loss of neuronal cell bodies [13], and allodynia [11]. Dynorphin A may directly bind to the NMDA receptors in vitro and inhibit the NMDA induced currents [10,16,17]. These inhibitory effects, however, contradict the excitatory and excitotoxic actions of dynorphin A in vivo and therefore do not support the hypothesis that the NMDA receptors are the primary non-opioid targets for dynorphin A. MNDA antagonists may block non-opioid effects of dynorphin A on more distal stages, for instance, preventing excitotoxic actions of excitatory amino acids released by this peptide [18,19]. Nonetheless, interactions with the glutamate receptors do not exclude the possibility of mediation of non-opioid effects of dynorphin A via distinct mechanisms that may be based on specific interactions of this peptide with other cellular systems.

In the present study, we attempted to identify non-opioid binding sites/factors for dynorphin A in neuronal cell lines, brain, and spinal cord, and to characterize the specificity and affinity of their interactions with dynorphins and other neuropeptides. A novel soluble factor that binds dynorphins A(1–17), A(1–13), and A(2–17) with high specificity and affinity has been found. Binding of dynorphin A to the factor was practically irreversible and resulted in conversion of dynorphin A into Leu-enkephalin, suggesting that the dynorphin A-binding factor (DABF) functions as an oligopeptidase that may be also involved in degradation of other neuropeptides.

## Materials and methods

*Materials.* Dynorphin A(1–17), dynorphin B(1–13), big dynorphin, dynorphin B, and central fragment of big dynorphin(6–26) were synthesized at the Department of Medical Biochemistry and Microbiology, University of Uppsala, purified by chromatography on Vydac C18 218 TP 1022 and Sephasil C8 columns in 0.1% trifluoroacetic acid (TFA)–acetonitrile and by gel filtration on a Superdex column in 1 M acetic acid, and analyzed by analytical reverse phase chromatography and MALDI-Tof. Purity was about 99%. Dynorphin A(1–10), dynorphin A(3–13), and dynorphin A(9–17) were purchased from Phoenix Pharmaceuticals, Karlsruhe. Dynorphin A(1–8), ($Arg^{11,13}$)-dynorphin A(1–13)-Gly-NH-$(CH_2)_5$-$NH_2$, Leu-enkephalin-$Arg^6$, Leu-enkephalin, pentalysine, α-melanocyte-stimulating hormone (α-MSH), corticotropin-releasing hormone (CRH), neurotensin, and substance P were purchased from Bachem, Bubendorf.

*Cell culture and tissue samples.* Human neuroblastoma SK-N-MC cells, choriocarcinoma JEG-3 cells, small cell lung carcinoma U-2020, U-1690, and U-1906 cells, and lymphoblast-like IM-9 cells were cultured in RPMI 1640, supplemented with 10% fetal calf serum, 100 IU penicillin, and 100 μg streptomycin per ml. NG 108-15 neuroblastoma X glioma hybrid cells were grown in Dulbecco's modified Eagle's medium and Ham's Nutrient Mixture F 12 containing calf serum (10%). Tissues were obtained from adult NMRI mice and 17-day-old embryos, 5-day-old pups, and adult Sprague–Dawley rats.

*Cell/tissue extracts.* All procedures were carried out on ice. Cells/tissue samples in 4 volumes of Dignam's buffer C (20 mM Hepes, pH 7.9, 0.42 M NaCl, 25% glycerol, 1.5 mM $MgCl_2$, 0.4 mM EDTA, and 0.5 mM DTT) supplemented with 0.2% NP-40 and protease inhibitors (0.5 mM PMSF, 10 μg/ml leupeptin, 0.1 mM aminobenzamidine, 10 g/ml aprotinin, and 1 μg/ml pepstatin A) were homogenized in a Dounce all-glass homogenizer, incubated for 10 min, and centrifuged twice for 10 min at 20,000*g* and the supernatant was kept at −80 °C. DTT was excluded from buffer C upon preparation of extracts for the *p*-hydroxy-mercuribenzoic acid (PHMB) and *N*-ethylmaleimide (NEM) studies. Protein concentrations were determined by the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA).

*Labeled peptide.* Peptides were labeled with $^{125}I$ and purified by HPLC as described [20], diluted in buffer D (0.05 M sodium phosphate, pH 7.4, 0.15 M NaCl, 0.02% sodium azide, 0.1% gelatin, 0.1% BSA, and 0.1% Triton X-100), and kept at −20 °C.

*Dynorphin A binding assay.* Binding reaction was carried out on ice or at 37 °C in buffer D in the presence of 1.5 mM DTT. Nearly identical results were obtained with Hepes buffer (0.02 M Hepes, pH 7.4, 0.1 M NaCl, 1 mM EDTA, 0.2% BSA, and 1.5 mM DTT). DTT was excluded from the buffer in experiments with PHMB and NEM. Cell/tissue extract (from 2 to 20 μg of protein, 10 μg in most experiments) and iodinated peptide (30,000 cpm) in buffer D or Hepes buffer were incubated for 30 min or longer as indicated in the figures. An equal volume of charcoal/dextran suspension (5 mg/ml charcoal and 5 mg/ml dextran T70 in 0.02 M Hepes buffer, pH 7.4, 0.1 M NaCl, and 0.5% BSA) was added and after 5 min incubation mixture was centrifuged in a Beckman microfuge for 5 min. In this procedure, unbound peptide was precipitated with charcoal, whereas labeled peptide that had formed complexes with extract components was retained in the supernatant.

*SP-Sephadex C-25 gel chromatography.* SK-N-MC extract was incubated with labeled dynorphin A (100,000 cpm), mixed with dextran/charcoal suspension, and centrifuged. The supernatant containing labeled peptide–protein complexes was mixed with an equal volume of 99% methanol–0.1% HCl at 4 °C, incubated for 30 min at 4 °C, centrifuged and the resulting supernatant diluted with 0.018 M pyridine and 0.1 M formic acid, pH 3.0, was loaded onto SP-Sephadex C-25 column. Peptides were eluted stepwise with 0.018 M pyridine and 0.1 M formic acid, pH 3.0 (fraction 1), 0.1 M pyridine and 0.1 M formic acid, pH 4.3 (elutes Leu-enkephalin; fraction 2), 0.35 M pyridine and 0.35 M formic acid, pH 4.3 (elutes Leu-enkephalin-$Arg^6$; fraction 3), 0.8 M pyridine and 0.8 M formic acid, pH 4.3 (fraction 4), and 1.6 M pyridine and 1.6 M formic acid, pH 4.3 (elutes dynorphin A, fraction 5) [20].

*Fast protein liquid chromatography.* SK-N-MC cell extract (0.9 mg protein) was applied at 4 °C on a Superose 6HR 10/30 column on fast protein liquid chromatography (FPLC)-system Pharmacia LKB FRAC-100, proteins were eluted with 20 mM Hepes buffer, pH 7.4, containing 0.15 M NaCl, and fractions were analyzed for the presence of dynorphin A-binding activity.

*Recombinant human thimet oligopeptidase.* Human thimet oligopeptidase (TOP) was prepared as described previously [21]. Briefly the coding sequence was subcloned into a pET32a (Novagen) expression vector and expressed in *Escherichia coli* BL21(DE3)RP cells (Stratagene) to overcome poor protein production caused by differences in codon usage. TOP was purified by metal affinity chromatography using Ni–NTA beads (Qiagen). The protein was eluted from the beads by incubation with enterokinase (Invitrogen) to cleave it from the polyhistidine containing N-terminal fusion sequence. The activity of the purified enzyme was determined by monitoring cleavage of a fluorogenic peptide substrate with the sequence of the 13-residue peptide neurotensin.

## Results

To test whether there are cellular components that interact specifically with dynorphin A we incubated

iodinated dynorphin A with extracts of neuroblastoma SK-N-MC or neuroblastoma X glioma NG 108-15 cell line. Dextran-coated charcoal was used to separate free and bound labeled peptide. After centrifugation unbound peptide was present in the charcoal pellet whereas radioactivity in the supernatant corresponded to the bound form of dynorphin A. Dynorphin A radioactivity in the supernatant was 2- to 10-fold higher in the presence of the SK-N-MC (Fig. 1) and NG 108-15 (not shown) cell extracts than in their absence. Preincubation of extracts with 1 μM unlabeled dynorphin A for 20 min reduced the level of bound peptide to levels observed in the absence of extract. Specific binding of labeled dynorphin A determined as the difference between the total binding and that in the presence of 1 μM unlabeled peptide showed a linear dependence on the concentration of labeled peptide in the range from 5000 to 250,000 cpm/tube. The dependence of specific binding on extract concentration was also linear in the range from 2 to 20 μg of extract protein/tube. The levels of specific binding did not change when time period of incubation of the reaction mixture with charcoal–dextran suspension was increased from 5 to 30 min, suggesting that the dissociation rate of dynorphin A from the complex is low. Iodinated dynorphin B and Leu-enkephalin-Arg$^6$ did not form complexes with SK-N-MC cell extract; the binding levels were the same in the absence and presence of extract and were not inhibited by excess of respective unlabeled peptide (Fig. 1).

Affinity and specificity of dynorphin A interactions with DABF were studied in competition experiments with dynorphins and longer prodynorphin fragments, other opioid and non-opioid peptides, and non-peptide ligands of opioid receptors. Preincubation of SK-N-MC or NG 108-15 cell extracts with dynorphin A(1–17), dynorphin A(2–17), dynorphin A(1–13), big dynorphin (BD), and a central BD fragment (BD6–26) concentration-dependently inhibited the specific binding of labeled dynorphin A (Fig. 2). Fifty percentage of inhibition was observed in the 5–10 nM range. Both (Arg$^{11,13}$)-dynorphin A(1–13)-Gly-NH-$(CH_2)_5$-$NH_2$ and dynorphin A(3–13) displaced 50% of labeled peptide at 50–100 nM concentrations. Dynorphin A fragments (1–10) and (6–12) displaced 50% of labeled peptide at $10^{-5}$ M concentration, whereas Leu-enkephalin, Leu-enkephalin-Arg$^6$, dynorphin A(1–8), and dynorphin A(9–17) at this and lower concentrations did not interfere with binding. Other opioid peptides including dynorphin B, α-neoendorphin, β-endorphin, Met-enkephalin, non-opioid peptides including α-MSH, CRH, substance P, neurotensin, or non-peptide ligands of opioid receptors such as morphine, etorphine, naloxone, bremazocine, and U50488, all at $10^{-5}$ M concentrations failed to displace labeled dynorphin A. $10^{-5}$ M pentalysine, which in similarity to dynorphin A is highly positively charged, failed to compete with labeled dynorphin A. These data demonstrate that the DABF binds dynorphin A with high affinity and specificity and that the epitope of dynorphin A critical for interactions with DABF comprises the dynorphin A(6–13) segment.

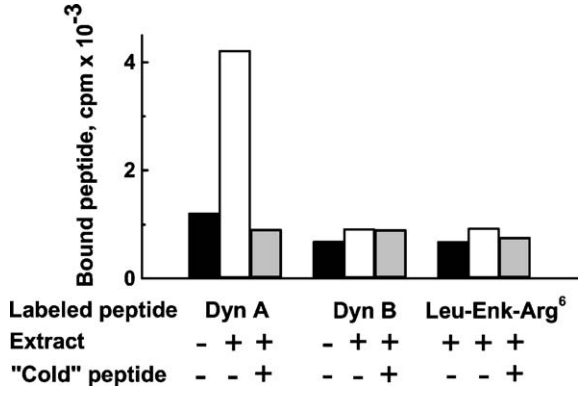


Fig. 1. Binding of labeled peptides to SK-N-MC cell extract. Unbound labeled peptide was adsorbed on dextran-coated charcoal and removed from the mixture by centrifugation. Peptide radioactivity in the supernatant is shown on the ordinate. In the competition experiments 1 μM respective "cold" peptide was added.

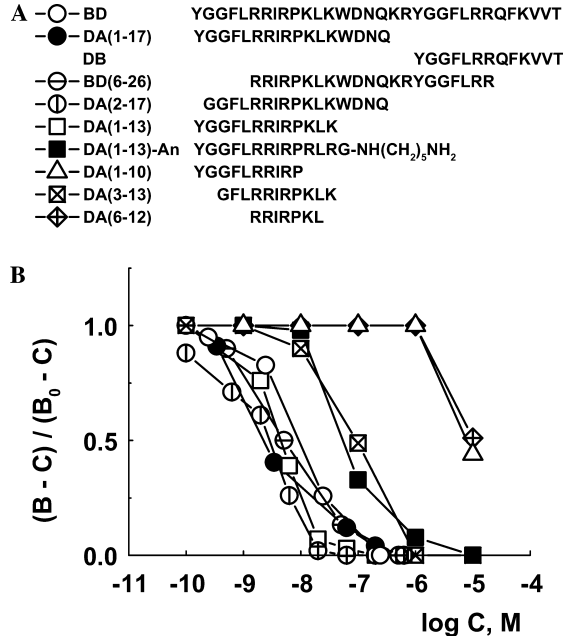


Fig. 2. (A) Peptides studied in competition experiments. (B) Competition of unlabeled dynorphins and their fragments with labeled dynorphin A for binding to the dynorphin A binding factor (DABF) in NG 108-15 cell extract. The same data were obtained with SK-N-MC cell extract. Ordinate $((B-C)/(B_0-C))$ gives the ratio of the binding levels in the presence $(B-C)$ and in the absence $(B_0-C)$ of competitors. $B_0$ and $B$ are the binding levels in the presence of extract whereas $C$ is background-binding levels in the absence of extract. Extract was preincubated with unlabeled peptides for 20 min, and after addition of labeled dynorphin A incubation continued for the next 30 min.

Binding of labeled dynorphin A to DABF in SK-N-MC cell extract reached the maximal level after 30 min of incubation and did not change during the next 4 h (Fig. 3A, curve 1). One micromolar unlabeled dynorphin A added to labeled dynorphin A preincubated with cell extract for 20 min did not displace labeled peptide from the preformed complex during the next 4 h (Fig. 3A, curve 2), demonstrating low dissociation rate.

The data shown on Figs. 1, 2, and 3A were obtained in the presence of DTT. When DTT was omitted from the buffer used for extract preparation and from the reaction medium, the binding levels were 2-fold lower. The SH group blockers PHMB (10 μM) and NEM (0.5 mM), added into the reaction medium prior to extract, completely abolished binding (Fig. 3B and some data not shown). However, PHMB added into the reaction medium after preincubation of labeled peptide with cell extract for 20 min did not change the concentration of dynorphin A–DABF complex during the next 4–5 h (Fig. 3B, curve 6). This again suggests a low rate of dissociation of dynorphin A–DABF complex.

To analyze the radioactive peptide present in the complex with DABF, labeled dynorphin A was incubated with SK-N-MC cell extract, and unbound peptide was precipitated by a dextran–charcoal mixture. The supernatant was mixed with methanol–HCl for dissociation of the complex and analyzed directly by SP-Sephadex C-25 chromatography (Fig. 4) or by 10% polyacrylamide gel electrophoresis after lyophilization (data not shown). Under these conditions, labeled dynorphin A, Leu-enkephalin, and Leu-enkephalin-Arg$^6$ were readily separated (Figs. 4A–C). Labeled peptide extracted from the complex was eluted in the same fraction (Fig. 4D) and demonstrated the same mobility on polyacrylamide gels (not shown) as labeled Leu-enkephalin, but not as dynorphin A and Leu-enkephalin-Arg$^6$. Thus, dynorphin A apparently undergoes conversion to its fragment Leu-enkephalin upon binding to or dissociation from DABF, suggesting that DABF has dynorphin A-specific oligopeptidase activity.

Size-exclusion chromatography of SK-N-MC cell and spinal cord extracts revealed a single peak of

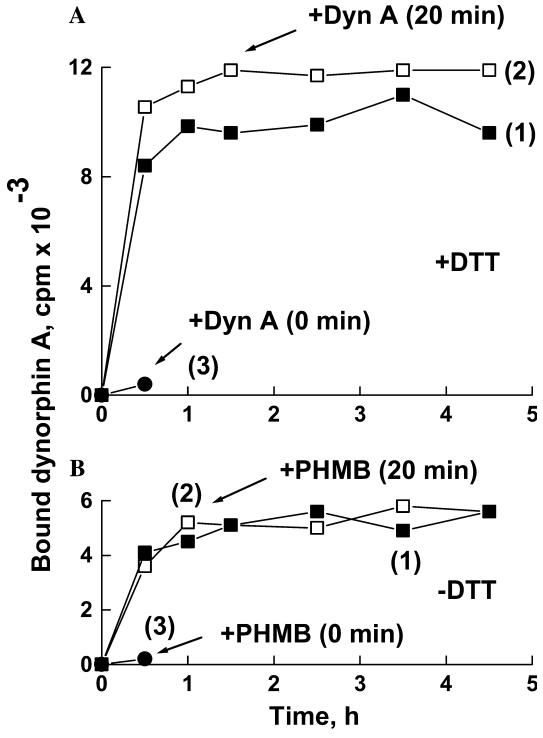


Fig. 3. Reversibility of labeled dynorphin A–DABF complex formation. Effects of DDT and PHMB. Reaction was carried out in the presence of 1.5 mM DDT (A) or in its absence (B). One micromolar unlabeled dynorphin A (A) or 10 μM PHMB (B) was added to the reaction mixture with SK-N-MC cell extract prior to labeled dynorphin A (A, curve 3, and B, curve 3) or 20 min after initiation of the binding reaction (A, curve 2, and B, curve 2). Curves 1 in A and B represent control measurements made with labeled peptide and extract only.

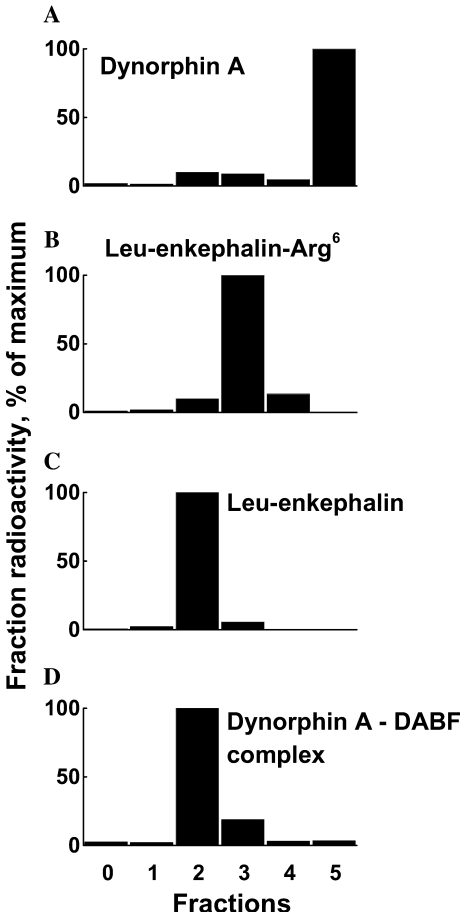


Fig. 4. SP-Sephadex C-25 chromatography of labeled peptide extracted from its complex with DABF. (A–C) Elution profile of labeled dynorphin A, Leu-enkephalin-Arg$^6$, and Leu-enkephalin, respectively. (D) Elution profile of labeled peptide extracted from dynorphin A–DABF complex. SK-N-MC cell extract was incubated with labeled dynorphin A, unbound peptide was precipitated by a charcoal/dextran mixture, and the supernatant mixed with 99% methanol–0.1% HCl, was loaded onto SP-Sephadex C-25 column.

dynorphin-binding activity with a molecular weight of about 70 kDa (Fig. 5), suggesting that this activity is associated with a single protein in the analyzed protein extracts.

Examination of molecular size, biochemical properties, and substrate specificity of known oligopeptidases brought our attention to the 78 kDa thimet oligopeptidase (TOP; EC3.4.24.15), that is involved in regulation of neuropeptide processing. TOP is activated by thiol reducing agents and demonstrates high affinity and low degradation rate for dynorphin A compared to other peptides [22,23]. To determine whether DABF is identical to TOP we tested if the recombinant enzyme binds dynorphin A and if the binding is specific for this peptide (Fig. 6). Whereas binding of dynorphin A to DABF from NG 108-15 cell extract was the same at 4 and 37 °C, recombinant TOP was able to bind this peptide at 37 °C but not 4 °C. Binding of labeled dynorphin A to TOP at 37 °C was inhibited by unlabeled dynorphin A and dynorphin B with equal efficiency; $IC_{50}$ values for both peptides were 250-fold higher for TOP than $IC_{50}$ for dynorphin A binding to DABF. These data demonstrate that DABF and TOP are different enzymes.

DABF levels were compared in adult rat and murine tissues and several tumor cell lines (Fig. 7). In adult rat brain, DABF activity was high in the periaqueductal gray (PAG), cortex, pituitary gland, and striatum, intermediate in dental gyrus (DG), hypothalamus, and cerebellum, and low in the ventral tegmental area (VTA). Dynorphins, on the other hand, are expressed at the highest levels in the pituitary gland, at lower levels in the nucleus accumbens, hippocampus, striatum, and VTA, whereas no peptides are found in the cerebellum [24–26]. DABF activity was present in all adult mouse tissues examined including the cerebrum, cerebellum, liver, and kidney. Thus, there is no correlation between the levels of DABF and dynorphin A.

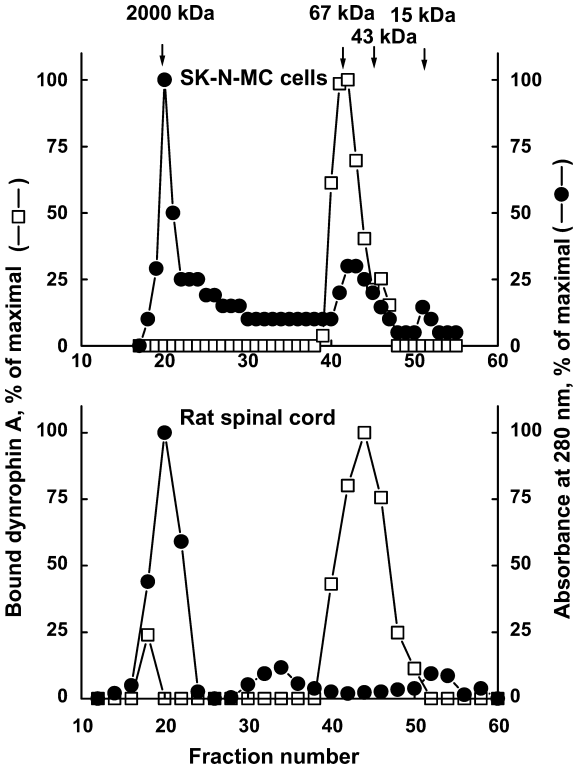


Fig. 5. Size-exclusion chromatography of DABF in SK-N-MC cell (A) and spinal cord (B) extract. After chromatography fractions were tested for the presence of dynorphin A-binding activity; aliquots were incubated with labeled dynorphin A following precipitation of unbound labeled peptide by dextran–charcoal. Ordinate shows fraction distribution of dynorphin A-binding activity.

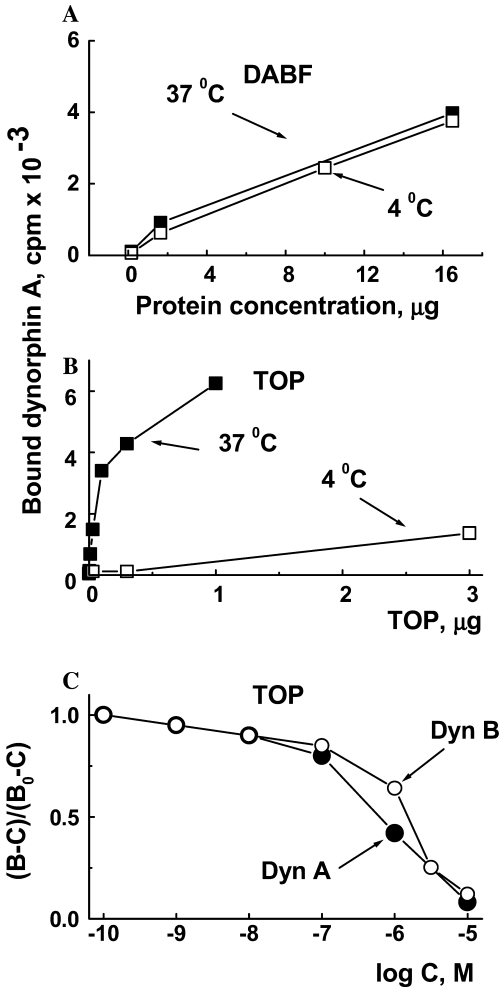


Fig. 6. TOP–dynorphin A interactions. Binding of labeled peptide to NG 108-15 cell extract (A) or TOP (B) at 37 and 4 °C. Peptide radioactivity in the supernatant after precipitation of unbound peptide by dextran–charcoal mixture is shown on the ordinate. (C) Competition of unlabeled dynorphin A and dynorphin B with labeled dynorphin A for interaction with TOP at 37 °C. Ordinate $((B-C)/(B_0-C))$ gives the ratio of the binding levels in the presence $(B-C)$ and in the absence $(B_0-C)$ of competitors. $B_0$ and $B$ are the binding levels in the presence of TOP whereas $C$ is background-binding levels in the absence of TOP. TOP was preincubated with unlabeled peptides for 20 min, and after addition of labeled dynorphin A incubation continued for the next 30 min.

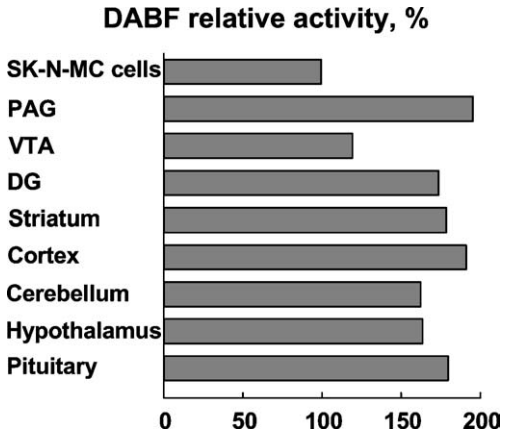


Fig. 7. Relative DABF levels in rat brain structures and pituitary gland. Binding to SK-N-MC extract was taken as 100%. Preincubation with 1 μM dynorphin A completely inhibited binding in all samples.

DABF was also found in all tumor cell lines analyzed with high levels in neuroblastoma SK-N-MC, hybrid neuroblastoma X glioma NG 108-15, choriocarcinoma JEG-3, and small cell lung carcinoma U-2020 cells and low levels in small cell lung carcinoma U-1690 and U-1906 cells and lymphoblast-like IM-9 cells.

## Discussion

A novel soluble non-opioid binding factor for dynorphin A(1–17), dynorphin A(2–17), and big dynorphin has been identified in the present study and characterized in neuronal and other cell lines and murine tissues. The factor is highly specific; only unlabeled dynorphin A and its versions that contain dynorphin A(6–13) segment inhibited binding of labeled dynorphin A to DABF. Other peptides including dynorphin B, endorphins, enkephalins, substance P, CRH, and non-peptide ligands of opioid receptors showed no binding. Dynorphin A interaction with DABF was virtually irreversible. The peptide extracted from the preformed dynorphin A–DABF complex was identified as Leu-enkephalin. These findings suggest that the binding of dynorphin A to, or its dissociation from, DABF results in conversion into Leu-enkephalin.

The binding epitope was mapped to dynorphin A(6–13) segment. However, comparison of dynorphin A(1–13) with dynorphin A(3–13) and BD(6–26) with dynorphin A(6–12) demonstrates that the N- and C-termini, respectively, contribute to the binding. Extension of the binding core (6–13) either from the N-terminus as in dynorphin A(1–13) or from the C-terminus as in BD(6–26) results in a similar increase in binding affinity, suggesting that overall peptide length but not N-terminal, C-terminal or central localization of the core (6–13) segment in a peptide determines binding affinity.

Similarity in molecular size, thiol dependence, and ability to hydrolyze oligopeptides suggested a DABF identity with thimet oligopeptidase (TOP), known to interact with dynorphin A with high specificity [22,23]. Experiments with recombinant TOP, however, did not support this suggestion but demonstrated that TOP may form complexes with dynorphin A corroborating the idea that DABF belongs to the same class of enzymes as TOP. The binding of peptide substrates to another oligopeptidase, insulysin, has also been demonstrated [27]. Binding of peptide substrates induces a conformational change in oligomeric insulysin that shifts the equilibrium to the more active enzyme forms suggesting a regulatory function of such binding.

Protease and proteasome inhibitors included in the extraction buffer or reaction medium (data not shown) did not interfere with dynorphin A–DABF interactions, demonstrating that DABF is not a serine (that is blocked by leupeptin, PMSF or aprotinin), an aspartic (that is blocked by pepstatin A) or a proteasome-associated (that is blocked by MG132) protease. Activation and inhibition of dynorphin A-binding activity by DTT and SH-group blockers, respectively, suggests that DABF is a cysteine oligopeptidase.

Membrane non-opioid dynorphin-binding sites on secretory vesicles of a pituitary-derived cell line have been reported [28]. These sites, however, are less specific than DABF; dynorphin A(1–17) and dynorphin A(2–17) compete with labeled dynorphin A for the pituitary sites with $IC_{50}$ values ranging from 0.2 to 2 μM. These values are 40- to 200-fold higher than respective values for DABF. The reversibility of binding was not evaluated. Considering high levels of DABF in the pituitary gland and partial association of this protein with particulate fractions (data not shown) we cannot rule out that dynorphin A-binding sites associated with secretory vesicles are identical to DABF molecules.

Cysteine oligopeptidases appear to play a critical role in the degradation of dynorphin A and its conversion into shorter enkephalins. Dynorphin A-converting enzymes purified from the spinal cord as a protein with a molecular mass of 50 kDa that cleaves dynorphin A into $Leu^5$-enkephalin-$Arg^6$ and $Leu^5$-enkephalin, or as a 65-kDa protein that cleaves dynorphin A to dynorphin A(1–8) were identified as cysteine or thiol-sensitive proteases [29–31]. High specificity for dynorphin A conversion suggested that these enzymes are involved in the generation of enkephalins, selective ligands for δ-opioid receptors from dynorphin A, that preferentially interacts with κ-opioid receptors. Degradation of dynorphin A in extracts of mouse spinal cord is inhibited by the cysteine protease inhibitor NEM [32]. Similarly, NEM and PHMB reduced the formation of Leu-enkephalin from dynorphin A(1–8) in slices of rat lumbosacral spinal cord [33]. We have recently demonstrated that i.t.-administered NEM, a cysteine protease inhibitor, produces

nociceptive behavior in wild type but not in prodynorphin knockout mice [32]. NEM also inhibited degradation of dynorphin A in spinal cord extracts, suggesting that NEM induces nociceptive behavior due to the inhibition of degradation/conversion of endogenous dynorphin A by cysteine proteases. In this regard, it is important to test whether DABF is involved in degradation of dynorphin A, a major pathogenic factor in chronic pain, or in its conversion into shorter enkephalins.

Another function of DABF may be to regulate the levels of dynorphin A available for interactions with the NMDA receptors by the sequestration of this peptide through formation of stable complexes. The stable dynorphin A–DABF complexes may also represent a transport form of this peptide or its fragments from the sites of dynorphin A release to the peptide cellular targets. However, the presence of DABF in tissues with low levels or absence of prodynorphin expression such as the cerebellum and liver does not support these ideas. DABF, as an oligopeptidase, may be involved in degradation of other neuropeptides, and thereby dynorphin A may inhibit this process by competition for binding to the DABF catalytic site.

Elucidating a role of DABF in non-opioid mechanisms mediating effects of dynorphin A in chronic pain is important for understanding the mechanisms of processing of nociceptive information in the spinal cord. Design of chemical compounds that can allosterically or by other mechanisms modulate DABF activity may provide new pharmacological tools to interfere with chronic pain.

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