Ca\(^{2+}\)-dependent activator proteins of secretion (CAPS) 1 and 2 are essential regulators of synaptic vesicle and large dense core vesicle priming in mammalian neurons and neuroendocrine cells. CAPS1 appears to have an additional and as yet unexplained function in vesicular catecholamine uptake or storage as CAPS1-deficient chromaffin cells exhibit strongly reduced vesicular catecholamine levels. Here we describe a role of CAPS proteins in vesicular monoamine uptake. Both CAPS1 and CAPS2 promote monoamine uptake and storage mediated by the vesicular monoamine transporters VMAT1 and VMAT2. Monoamine uptake of vesicular preparations from embryonic brains of CAPS1 deletion mutants is decreased as compared with corresponding preparations from wild type littermates, and anti-CAPS1 or anti-CAPS2 antibodies inhibit monoamine sequestration by synaptic vesicles from adult mouse brain. In addition, overexpression of CAPS1 or CAPS2 enhances vesicular monoamine uptake in Chinese hamster ovary cells that stably express VMAT1 or VMAT2. CAPS function has been linked to the heterotrimeric GTPase Go, which modulates vesicular monoamine uptake. We found that the expression of CAPS1 is decreased in brain membrane preparations from mice lacking Go\(_{\alpha}\), which may explain the reduced monoamine uptake by Go\(_{\alpha}\)-deficient synaptic vesicles. Accordingly, anti-CAPS1 antibodies do not further reduce monoamine uptake by Go\(_{\alpha}\)-deficient synaptic vesicles, whereas antibodies directed against CAPS2, whose expression is not altered in Go\(_{\alpha}\)-deficient brain, still reduce monoamine uptake into Go\(_{\alpha}\)-deficient vesicles. We conclude that CAPS proteins are involved in optimizing vesicular monoamine uptake and storage mediated by VMAT1 and VMAT2.

Secretion of neurotransmitters is mediated by Ca\(^{2+}\)-dependent fusion of secretory organelles with the plasma membrane. CAPS proteins were initially identified as cytosolic regulators of Ca\(^{2+}\)-dependent secretion in PC12 cells (1). Mammals express two isoforms of CAPS, CAPS1 and CAPS2, which have similar functions but differ in their spatiotemporal expression pattern (1–7). Subsequent studies on mammalian CAPS isoforms showed that CAPS proteins play an essential role in the priming step of synaptic vesicles and large dense core vesicles (1, 2, 7–10). In addition, CAPS1 appears to have a second and as yet unexplained function in vesicular catecholamine uptake or storage (10).

Transport of monoamines into secretory vesicles is mediated by VMATs. In mammals, two isoforms, termed VMAT1 and VMAT2, have been identified (11, 12). VMAT1 expression is restricted to neuroendocrine cells, and VMAT2 is the only isoform expressed in neurons. Monoamine uptake via VMAT1 and VMAT2 is regulated by vesicle-associated heterotrimeric G-proteins, which is illustrated by the fact that in neurons and neuroendocrine cells, the \(\alpha\)-subunit of Go\(_{\alpha}\) inhibits monoamine uptake (13–15). The role of CAPS proteins in VMAT-mediated vesicular catecholamine uptake and storage is currently discussed controversially because corresponding studies undertaken so far have yielded contradictory results. Experiments on chromaffin cells of CAPS1-deficient mice indicated a role of CAPS1 in catecholamine loading as CAPS1-deficient chromaffin cells exhibit strongly reduced catecholamine levels in their secretory granules. Indeed, many fusing chromaffin granules of CAPS1-deficient chromaffin cells contain no catecholamines at all (10). Chromaffin cells of mice lacking both CAPS1 and CAPS2 exhibit an additional reduction in the refilling and maintenance of a pool of rapidly releasable chromaffin granules but no further reduction in vesicular catecholamine sequestration as compared with chromaffin cells of mice lacking only CAPS1 (16). In contrast to these findings, knock-down of CAPS1 expression in PC12 cells was found to cause increased endogenous catecholamine levels and enhanced accumulation of norepinephrine (17). Based on these indirect readouts, a role of CAPS proteins in vesicular monoamine uptake was categorically excluded (17).

In the study reported here, we directly examined the role of CAPS proteins in vesicular monoamine uptake and storage employing a set of complementary experimental approaches. Our results support the notion that CAPS proteins promote vesicular monoamine uptake and storage in brain cells and engineered cell lines.

---

\* This work was supported by Max Planck Society Grant AH 67/3-3 (to G. A.-H.) and German Research Foundation Grant SFB406/A1 (to N. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\1 Present address: Lund University, Dept. of Clinical Sciences Malmö, UMAS Ing 72, CRC 91-11, S-20502 Malmö, Sweden.

\2 To whom correspondence should be addressed. E-mail: gudrun.ahnert@charite.de.

\3 The abbreviations used are: CAPS, Ca\(^{2+}\)-dependent activator protein of secretion; CH0, Chinese hamster ovary; VMAT, vesicular monoamine trans-
were densitometrically quantified using the Labimage 1D program (KAPELAN, Halle, Germany). It was ensured that signals were in the linear range of the ECL detection system. Quantification of proteins comparing wild type and knock-out samples were performed from the same gel using actin as internal standard.

**CHOVMAT1 and CHOVMAT2 Cell Lines**—VMAT1-expressing CHO cells were cultured at 37 °C, 5% CO₂ in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. CHOVMAT2 cells were cultured in the same medium supplemented with 400 μg/ml Geneticin. Cells were transfected with CAPS-DNA using the LipofectamineTM transfection reagent (Invitrogen). 2 days after transfection, cells were analyzed by fluorescence microscopy and serotonin uptake assays. After selective permeabilization of plasma membranes by SLO, serotonin uptake into intracellular compartments by VMAT1 or VMAT2 was assayed as described (21).

**Fluorescence Microscopy**—Cells were grown on glass cover-slips, washed twice with phosphate-buffered saline, and fixed in 4% formaline in 0.1 m phosphate buffer, pH 7.4, for 45 min at room temperature. After three rinses with phosphate-buffered saline and one rinse with water, coverslips were mounted on glass slides for fluorescence microscopic analysis.

**Experimental Design**—All experiments presented were repeated at least two times. Individual uptake experiments were performed in triplicate. Values are presented as mean ± S.D.

**RESULTS**

**Absence of CAPS Proteins Decreases Vesicular Serotonin Uptake**—Electrophysiological evidence indicates that the deletion of CAPS1 reduces vesicular monoamine uptake or storage in chromaffin granules (10). Efforts to obtain direct biochemical evidence for an effect of the CAPS1 deletion on vesicular monoamine uptake has been confounded by the fact that CAPS-deficient mice die at birth and thus yield only very small amounts of chromaffin granules that are not sufficient for biochemical uptake assays. To circumvent this problem, we tested first whether monoamine uptake assays into synaptic vesicles prepared from E18 embryonic mouse brains are feasible.

Using E18 embryonic synaptic vesicles, we found that serotonin uptake was 3-fold higher without additives as compared with uptake measured in the presence of 6 μM of the VMAT blocker reserpine, indicating a specific uptake via VMAT2 (Fig. 1A, left panel). This allowed us to analyze monoamine uptake by synaptic vesicles of CAPS1 deletion mutants prepared at E18. Due to the low uptake seen in samples from embryonic brains, serotonin uptake assays using vesicular preparations from embryonic brains of wild type, CAPS1+/−, and CAPS1−/− mice were performed with 400 nm serotonin instead of 40 nm, resulting in an increased uptake in general. Serotonin uptake was significantly decreased in vesicles from CAPS1−/− mice as compared with wild type littermates (Fig. 1A, right panel). Unspecific accumulation of serotonin in the presence of reserpine was comparable for all genotypes and subtracted (see the legend for Fig. 1A). Vesicles from heterozygous mice did not exhibit a significant reduction of serotonin uptake. The
CAPS Proteins Promote Monoamine Uptake

**FIGURE 1. Influence of CAPS1 and CAPS2 on serotonin uptake by synaptic vesicles.** A, left panel, serotonin uptake by a vesicular preparation from embryonic mouse brains (E18). Uptake in the absence of reserpine is 3-fold higher than in the presence of reserpine, indicating a specific accumulation of serotonin via VMAT2. Uptake assays were performed using 40 nM [3H]serotonin. Right panel, serotonin uptake was assayed using vesicular preparations from embryonic brains of wild type (wt), CAPS1+/−, and CAPS1−/− mice in the presence of 400 nM serotonin (40 nM [3H]serotonin plus 360 nM serotonin), accounting for higher accumulation of serotonin. Serotonin uptake was significantly decreased in CAPS1−/− samples. The reduction in uptake by preparations from CAPS1−/− mice was not statistically significant. Unspecific uptake determined in the presence of 6 μM reserpine was similar in wild type (2.80 pmol/mg of protein), CAPS1+/− (2.63 pmol/mg of protein), and CAPS1−/− samples (2.73 pmol/mg of protein) and was subtracted from total uptake values. Each experiment was repeated at least twice. The values given represent the means of three samples ± S.D. B, vesicular preparations from brains of embryonic wild type and CAPS1−/− littermates were analyzed by Western blotting using anti-VMAT2 and anti-actin antibodies. 5, 10, and 15 μg of protein of the respective sample were loaded. Quantification is given as relative optical density (OD) and was assessed using actin as an internal control. Values represent the mean of four animals per genotype ± S.D. C, mouse synaptic vesicles were incubated with anti-CAPS1 antibodies (left panel) or anti-CAPS2 antibodies (right panel) and the respective preimmune sera (PIS) for 30 min on ice. The serotonin uptake assay was then performed as described above. Incubation with both anti-CAPS1 and anti-CAPS2 antibodies decreased vesicular serotonin uptake significantly. D, serotonin uptake was assayed using vesicular preparations from embryonic brains of wild type and CAPS1−/− mice using 400 nM serotonin as given in B. Serotonin uptake was significantly decreased in CAPS1−/− samples but was not further decreased by application of the anti-CAPS1 antibody, which, however, decreased uptake into vesicular preparations from wild type mice. The values given are corrected for unspecific accumulation in the presence of reserpine and represent the means of three samples ± S.D.

Encouraged by these data, we tested next whether a reduction of uptake can also be seen when CAPS protein function in synaptic vesicles from adult brain is perturbed by specific antibodies. Synaptic vesicles were incubated with anti-CAPS1 or anti-CAPS2 antibodies and the respective preimmune sera prior to the uptake procedure. Although preincubation with preimmune sera showed no effect, incubation with both anti-CAPS antibodies reduced serotonin uptake by 40% (Fig. 1C). To demonstrate the specificity of the antibodies used in the perturbation assay, vesicular preparations from CAPS1−/− and the corresponding wild type embryonic brains were incubated with the anti-CAPS1 antibody. As expected, a decrease in the uptake by the anti-CAPS1 antibody was only observed in the wild type but not in the CAPS1−/− preparation (Fig. 1D). Uptake into CAPS1−/− vesicles was reduced as compared with wild type vesicles, confirming the effect of the CAPS1 mutation seen before. These results indicate that both CAPS isoforms influence monoamine uptake and/or storage in a similar manner and that the reduced uptake activity seen in vesicles from CAPS1-deficient brain is most likely a direct effect of the CAPS1 loss and not due to a secondary developmental or homeostatic effect.

Expression of CAPS1 Is Decreased in Brains of Go2α−/− Mice—Both CAPS1 and the α-subunit of heterotrimeric G-protein Go2 are involved in monoamine uptake and storage (10, 24), and an indirect functional link between Go proteins and the CAPS ortholog Unc-31 in Caenorhabditis elegans was reported previously (25). Thus, we analyzed the expression of CAPS1 and CAPS2 in brain fractions of wild type and Go2α−/− mice. CAPS1 levels were significantly reduced in homogenates and synaptosomal fractions of Go2α−/− deletion mutants as compared with wild type littermates (Fig. 2A), whereas CAPS2 levels were similar in brains from both genotypes (Fig. 2B).

Subsequently, the effects of anti-CAPS1 and anti-CAPS2 antibodies on serotonin uptake of synaptic vesicles from Go2α−/− mice were determined and compared with the effect induced in vesicles of wild type mice. Specificity of the antibodies was demonstrated previously (7, 10) (Fig. 1D). In accordance with the lower expression levels of CAPS1 in Go2α−/− mutants, there was no effect of anti-CAPS1 antibodies on vesicular serotonin uptake into Go2α−/− vesicles (Fig. 2C, left panel). In contrast, the effect of anti-CAPS2 antibodies on serotonin uptake was not further decreased by application of the anti-CAPS1 antibody, which, however, decreased uptake into vesicular preparations from wild type mice. The values given are corrected for unspecific accumulation in the presence of reserpine and represent the means of three samples ± S.D.
on synaptic vesicles from G_{o_2}/a^{−/−} mice further confirms the specificity of the antibodies used. Generally, serotonin uptake was lower in synaptic vesicles from G_{o_2}/a^{−/−} animals as compared with vesicles of wild type animals, as observed previously, although VMAT expression is increased in G_{o_2}/a^{−/−} mice (15). Probably, the reduced expression of CAPS1 accounts for the reduced monoamine uptake in vesicles of G_{o_2}/a^{−/−} mice.

Transfection of VMAT-expressing CHO Cells with CAPS1 and CAPS2 Increases Vesicular Serotonin Uptake—The data reported above show that both genetic deletion of CAPS and perturbation of CAPS function by specific antibodies reduce monoamine uptake into synaptic vesicles. To see whether increased CAPS function leads to the opposite effect, i.e., increased vesicular monoamine uptake, CHO cell lines that permanently express either VMAT1 or VMAT2 were transiently transfected with CAPS-encoding cDNAs and assayed for VMAT activity. These VMAT-expressing cell lines are devoid of plasma membrane monoamine transporters and monoamine-synthesizing or -degrading enzymes, and VMATs were shown to reside on endosomal compartments when expressed in CHO cells (24). After permeabilization of the plasma membrane, this model system is well suited for studies on vesicular monoamine uptake and its regulation (21).

VMAT1- and VMAT2-expressing CHO cells were transiently transfected with CAPS1 or CAPS2 cDNA. The respective CAPS sequences were placed in front of an IRES-eGFP sequence for fluorescence analysis of transfection efficiency. An IRES-eGFP-vector cDNA was used for control transfections. Kinetics of serotonin transport by VMAT1 and VMAT2 were analyzed following SLO permeabilization in CAPS1- (Fig. 3A) or CAPS2-transfected (Fig. 3B) CHOVMAT cells in comparison with vector-transfected cells. Transfection with CAPS1 significantly increased V_{max} values of VMAT1 and VMAT2 expressed in CHO cells, in comparison with IRES-eGFP-transfected cells (Fig. 3A), whereas K_{m} values remained unchanged. Likewise, transfection with CAPS2 cDNA led to an increase of V_{max} values for both VMAT isoforms with almost no change in K_{m} values (Fig. 3B and Table 1). These results confirm the notion that CAPS1 and CAPS2 promote vesicular monoamine uptake and/or storage.

**DISCUSSION**

Our results show that both CAPS isoforms promote vesicular monoamine uptake, irrespective of the VMAT isoform expressed. This function is fulfilled in both neuronal and non-neuronal cells and detectable in vesicle preparations from embryonic and adult mouse brain. The results are consistent with prior observations indicating a requirement of CAPS for filling of chromaffin granules (10, 16). They also reveal a functional link between G_{o_2}/a, which was shown previously to regulate monoamine storage, and CAPS1 as CAPS1 expression is decreased in brains of G_{o_2}/a^{−/−} mice.

Role of CAPS1 and CAPS2 in Vesicular Monoamine Loading—Both CAPS isoforms were shown to regulate large dense core vesicle and synaptic vesicle secretion in mammalian cells (8, 9). Previous investigations of an additional role of CAPS proteins in vesicular monoamine uptake and storage yielded contradictory results (10, 17). Our present results are in line

FIGURE 2. CAPS1 and CAPS2 in G_{o_2}/a^{−/−} mice. Subcellular fractions prepared from wild type (wt) and G_{o_2}/a^{−/−} brains were analyzed by Western blotting using anti-CAPS1 and anti-CAPS2 antibodies. A. CAPS1 levels are significantly reduced in brain homogenate and synaptosomes of G_{o_2}/a^{−/−} mice. The graphs show quantifications of lanes loaded with 10 μg of protein. Values represent the mean of four animals per genotype ± S.D. B, CAPS2 expression is not altered in brains of G_{o_2}/a^{−/−} mice. The graphs show quantifications of lanes loaded with 10 μg of protein. Values represent the mean of four animals per genotype ± S.D. C, crude synaptic vesicles of wild type and G_{o_2}/a^{−/−} mice were incubated with anti-CAPS1 antibodies (left panel, final dilution 1:20) or anti-CAPS2 antibodies (right panel, final dilution 1:20) and the respective preimmune sera (PIS). Afterward, serotonin uptake was assayed as described above. Left panel, in contrast to the results obtained with wild type samples, incubation with anti-CAPS1 antibodies did not change vesicular serotonin accumulation in G_{o_2}/a^{−/−} samples, probably due to diminished expression of CAPS1 in G_{o_2}/a^{−/−} mutants. Right panel, like in wild type animals, anti-CAPS2 antibodies decreased vesicular serotonin uptake in G_{o_2}/a^{−/−} mice. Although CAPS2-expression is unchanged in G_{o_2}/a^{−/−} as compared with wild type mice, the effect of anti-CAPS2 antibodies on vesicular serotonin uptake is even more pronounced in G_{o_2}/a^{−/−} samples than in wild type samples.
CAPS Proteins Promote Monoamine Uptake

FIGURE 3. Overexpression of CAPS1 or CAPS2 in VMAT-expressing CHO cells increases vesicular serotonin uptake. CHO cells expressing either VMAT1 or VMAT2 were transfected with eukaryotic expression vectors encoding rat CAPS1 (A) or human CAPS2 (B) and eGFP via an IRES-eGFP sequence. An IRES-eGFP-vector DNA was used for control transfections. Fluorescence analyses demonstrated efficient transfection of both constructs in each of the VMAT cell lines. Kinetics of serotonin transport by VMAT1 and VMAT2 were analyzed in CAPS1- or CAPS2-transfected CHOVMAT cells, in comparison with vector-transfected cells following SLO permeabilization. Uptake was assayed for 10 min at 37 °C using potassium-glutamate-ATP buffer containing 40 nM [3H]serotonin and increasing concentrations of unlabeled serotonin. Each experiment was repeated at least twice. Values represent means of three samples ± S.D. Asterisks denote statistical significant differences according to Student’s t-test (p < 0.05).

TABLE 1

Effects of CAPS1 and CAPS2 on VMAT1 and VMAT2 uptake kinetics

|          | VMAT1          | VMAT2          |
|----------|----------------|----------------|
|          | eGFP | CAPS1 | eGFP | CAPS2 |
| $K_m$ ($\mu$M) | 26.3 ± 11.2 | 36.7 ± 11.1 | 37.5 ± 9.8 | 34.4 ± 11.6 |
| $v_{max}$ (pmol/mg protein/10 min) | 208.3 ± 27.1 | 302.3 ± 32.4* | 391.8 ± 36.6 | 583.6 ± 67.8* |
| $v_{max}$ (pmol/mg protein/10 min) | 419.2 ± 31.8 | 593.8 ± 38.4* | 153.1 ± 50.0 | 247.3 ± 7.3* |

with one of these earlier studies, which demonstrated a facilitating role of CAPS in large dense core vesicle loading in mouse chromaffin cells (10, 16). In adrenal chromaffin cells from embryonic CAPS1$^{-/-}$ mutants, up to 70% empty (i.e. catecholamine-free) chromaffin granules were shown to undergo exocytosis. Additionally, cytosolic levels of catecholamine metabolites were found to be strongly increased in adrenal glands of CAPS1$^{-/-}$ mice, indicating a shift from vesicular to cytosolic catecholamine pools. These observations were interpreted in the context of a dysfunction of vesicular catecholamine loading or storage in the absence of CAPS1 (10). A recent study on chromaffin cells of deletion mutant mice lacking both CAPS isoforms also suggested, besides a strong deficit in exocytosis, fusion of empty vesicles (16). However, knock-down of CAPS1 in PC12 cells led to a higher accumulation of exogenous norepinephrine, and the endogenous level of dopamine was increased (17). Although catecholamine filling of secretory vesicles was not tested in this study, e.g. by directly correlating high resolution membrane fusion measurements with amperometric recordings of released catecholamines as was done in the conflicting study (10, 16), the phenomenon of increased norepinephrine accumulation and endogenous dopamine levels after CAPS1 knock-down was attributed solely to a reduction of (constitutive) secretion in CAPS1 knock-down cells. An additional direct role of CAPS proteins in vesicular monoamine uptake was excluded categorically (17). However, the endogenous amounts of dopamine and norepinephrine do not correlate in the various knock-down clones (i.e. high levels of dopamine were found associated with lower levels of norepinephrine and vice versa), whereas comparable amounts were seen in the control cells (17). Thus, it is possible that knock-down of CAPS1 also affects metabolism of dopamine or its turnover to norepinephrine. A change in the metabolic balance may also explain the selectively reduced amounts of dopamine as compared with the almost unchanged amounts of norepinephrine in the CAPS1$^{-/-}$ chromaffin cells (10). Essentially, without direct information on vesicular uptake characteristics, changes in the metabolic balance of dopamine and its metabolites can only be used as a very indirect readout for vesicular uptake, especially in a cell culture system such as PC12 cells.

Our present study demonstrates that both CAPS isoforms promote vesicular monoamine uptake and storage in both VMAT1-expressing and VMAT2-expressing cells (Figs. 1–3), thus supporting the data obtained in chromaffin cells of CAPS deletion mutants (10, 16). Overexpression of CAPS isoforms in CHOVMAT cells leads to effects, i.e. increased monoamine uptake (Fig. 3), that are opposite to those seen upon CAPS deficiency in deletion mutants (Fig. 1A) or after
CAPS Proteins Promote Monoamine Uptake

loss of function due to antibody-mediated perturbation (Fig. 1C). In view of these findings and the fact that neither VMAT2 nor vesicular proton pump levels are changed in CAPS1-deficient vesicles (Fig. 1B), we conclude that CAPS proteins positively regulate monoamine uptake and that the reduced vesicular monoamine uptake in CAPS-deficient cells is a direct consequence of CAPS loss rather than a secondary, indirect effect of the mutation. CAPS proteins may stimulate synaptic vesicles, allowing them to store higher amounts of monoamines by shifting the electrical proton gradient (ΔμH⁺) across the vesicle membrane to higher values. An increase of the ΔpH component of ΔμH⁺ would allow more monoamines to be stored inside vesicles and would counteract nonselective leakage (24, 26). However, the protein levels of the proton pump is not affected in CAPS1−/− vesicle preparations, and the loading of glutamatergic synaptic vesicles is only weakly affected by deletion of CAPS1 and CAPS2 (8), which indicates that the electrochemical gradient is functional in CAPS-deficient vesicles and that CAPS proteins preferentially regulate storage of monoamines in VMAT2-containing vesicles.

Functional Relationship between CAPS1 and Go2α—The α-subunit of Gα2 was shown to regulate vesicular monoamine storage. Vesicular monoamine uptake is decreased in Gα2α−/− mice (15) (Fig. 2C). Thus, both CAPS proteins and Gα2 are involved in vesicular monoamine loading. In addition, loss-of-function mutation of the heterotrimeric Gα protein of C. elegans partly suppresses the phenotypic deficits of unc-31 (CAPS) mutants, which further indicates a functional interplay between Unc-31/CAPS and Go2 mutants, which further indicates a functional interplay between Unc-31/CAPS and Go2−/− mice (2, 7). In the midbrain, CAPS2 is concentrated in dopaminergic nigro-striatal dopamine system was previously shown to be disturbed in Go2−/− mice. An increase of the H11002/H9262 chemical gradient is functional in CAPS-deficient vesicles and that CAPS proteins preferentially regulate storage of monoamines in VMAT2-containing vesicles.

Acknowledgments—We thank Carsten Enk (Göttingen, Germany) and Thomas F. J. Martin (Madison, WI) for generously providing CAPS cDNA vectors. The expert technical assistance of Birgit Metze (Charité) is gratefully acknowledged.

REFERENCES
1. Walentin, J. H., Porter, B. W., and Martin, T. F. J. (1992) Cell 70, 765–775
2. Hay, J. C., and Martin, T. F. J. (1992) J. Cell Biol. 119, 139–151
3. Ann, K., Kowalchyk, J. A., Loyet, K. M., and Martin, T. F. J. (1997) J. Biol. Chem. 272, 19637–19640
4. Cisternas, F. A., Vincent, J. B., Scherer, S. W., and Ray, P. N. (2003) Genomics 81, 279–2913
5. Sadakata, T., Itakura, M., Kozaki, S., Sekine, Y., Takahashi, M., and Furuiuchi, T. (2006) J. Comp. Neurol. 495, 735–753
6. Sadakata, T., Washida, M., Morita, N., and Furuiuchi, T. (2007) J. Histochem. Cytochem. 55, 301–311
7. Speidel, D., Varoquesaux, F., Enk, C., Nojiri, M., Grishanin, R. N., Martin, T. F. J., Hofmann, K., Brose, N., and Reims, K. (2003) J. Biol. Chem. 278, 52802–52809
8. Jockusch, W. J., Speidel, D., Sigler, A., Sørensen, J. B., Varoquesaux, F., Reims, J.-S., and Brose, N. (2007) Cell 131, 796–808
9. Grishanin, R. J., Kowalchyk, J. A., Klench, V. A., Kyousko, A., Earles, C. A., Chapman, E. A., Gerona, R. L. R., and Martin, T. F. J. (2004) Neuron 43, 551–562
10. Speidel, D., Brueederle, C. E., Enk, C., Voets, T., Varoquesaux, F., Reims, K., Becherer, U., Fornai, F., Ruggieri, S., Holghaus, Y., Weihe, E., Bruns, D., Brose, N., and Rettig, J. (2005) Neuron 46, 75–88
11. Liu, Y., Peter, D., Rhogani, A., Schulden, S., Prive, G. G., Eisenberg, D., Brecha, N., and Edwards, R. H. (1992) Cell 70, 539–551
12. Erickson, J. D., Eiden, L. E., and Hoffman, B. J. (1992) Proc. Natl. Acad. Sci.
CAPS Proteins Promote Monoamine Uptake

13. Höltje, M., von Jagow, B., Pahner, I., Lautenschlager, M., Hörttnagl, H., Nürnberg, B., Jahn, R., and Ahnert-Hilger, G. (2000) J. Neurosci. 20, 2131–2141
14. Pahner, I., Höltje, M., Winter, S., Nürnberg, B., Ottersen, O. P., and Ahnert-Hilger, G. (2002) Eur. J. Cell Biol. 81, 449–456
15. Brunk, I., Blex, C., Sanchis-Segura, C., Sternberg, J., Perreau-Lenz, S., Bilbao, A., Hörttnagl, H., Baron, J., Juranek, J., Laube, G., Birnbaumer, L., Spanel, R., and Ahnert-Hilger G. (2008) FASEB J. 22, 3736–3746
16. Liu, Y., Schirra, C., Stevens, D. R., Matti, U., Speidel, D., Hof, D., Bruns, D., Brosse, N., and Retig, J. (2008) J. Neurosci. 28, 5594–5601
17. Fujita, Y., Xu, A., Xie, L., Arubachalam, L., Chou, T.-C., Jiang, T., Chiew, S.-K., Kourtesis, J., Wang, L., Gaisano, H. Y., and Sugita, S. (2007) J. Biol. Chem. 282, 21392–21403
18. Weller, U., Müller, L., Messner, M., Palmer, M., Valeya, A., Tranum-Jensen, J., Agrawal, P., Biemann, C., Dobereiner, A., Kehoe, M. A., and Bhakdi, S. (1996) Eur. J. Biochem. 236, 34–39
19. Jiang, M., Gold, M. S., Boulay, G., Spicher, K., Pexton, M., Brabet, P., Srinivasan, Y., Rudolph, U., Ellison, G., and Birnbaumer, L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 3269–3274
20. Dhinra, A., Jiang, M. S., Wang, T. L., Lyubarsky, A., Savchenko, A., Bar-Yehudopamine, T., Sterling, P., Birnbaumer, L., and Vardi, N. (2002) J. Neurosci. 22, 4878–4884
21. Brunk, I., Blex, C., Rachakonda, S., Höltje, M., Winter, S., Pahner, I., Walther, D. J., and Ahnert-Hilger, G. (2006) J. Biol. Chem. 281, 33373–33385
22. Huttner, W. B., Schiebler, W., Greengard, P., and DeCamilli, P. (1983) J. Cell Biol. 96, 1374–1388
23. Becher, A., Drenckhahn, A., Pahner, I., Margittai, M., Jahn, R., and Ahnert-Hilger, G. (1999) J. Neurosci. 19, 1922–1931
24. Brunk, I., Höltje, M., von Jagow, B., Winter, S., Sternberg, J., Blex, C., Pahner, I., and Ahnert-Hilger, G. (2006) Handb. Exp. Pharmacol. 175, 305–332
25. Charlie, N. K., Schade, M. A., Thomure, A. M., and Miller, K. G. (2006) Genetics 172, 943–961
26. Edwards, R. H. (2007) Neuron 55, 835–858