in dopamine-rich areas of the S brain the activity of G/F protein may be increased or its coupling with adenylate cyclase facilitated.

The increase in responsiveness of the D_1 receptors located in the S brain could involve an increase in the number of G/F protein molecules operative in adenylate cyclase stimulation or an increase in the affinity of G/F protein for guanylimidodiphosphate. An increase in affinity is suggested by the observation that in homogenates of nucleus caudatus from S subjects the concentration of guanylimidodiphosphate needed to elicit half-maximum stimulation of adenylate cyclase is only 1/2 to 1/7 of that required by homogenates from nucleus caudatus of NS subjects. Such an increased affinity may cause a greater efficiency in the function of the G/F protein and in turn may facilitate the coupling of dopamine recognition sites with adenylate cyclase. In conclusion, the present data suggest that the enhanced dopamine function believed to be associated with schizophrenia may be the expression of an increased efficiency in the G/F coupling operative in linking D_1 recognition sites to adenylate cyclase.

MAURIZIO MEMO
Section on Biochemical Pharmacology, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205
JOEL E. KLEINMAN
Adult Psychiatry Branch, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032
INGEBORG HANBAUER
Section on Biochemical Pharmacology, National Heart, Lung, and Blood Institute

References and Notes

1. S. S. Kety, *Science* **129**, 1528 (1959).
2. S. H. Snyder, S. P. Banerjee, H. I. Yamamura, D. Greenberg, *ibid.* **184**, 1243 (1974).
3. S. Matthysse and J. Lipinski, *Annu. Rev. Med.* **26**, 551 (1975).
4. P. H. Connel, *Amphetamine Psychosis* (Oxford Univ. Press, London, 1958).
5. J. D. Griffith, J. H. Caranaugh, J. Held, in *Amphetamines and Related Compounds*, E. Costa and S. Garattini, Eds. (Raven, New York, 1969), p. 897.
6. J. M. Davis, *J. Psychiatr. Res.* **11**, 25 (1974).
7. I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976).
8. T. J. Crow *et al.*, *Br. J. Psychiatry* **134**, 249 (1979).
9. F. Owen *et al.*, *Lancet* **1978-II**, 223 (1978).
10. A. Carenzi *et al.*, *Arch. Gen. Psychiatry* **32**, 1056 (1975).
11. T. Lee and P. Seeman, *Am. J. Psychiatry* **137**, 191 (1980).
12. T. J. Crow, E. C. Johnstone, A. J. Longden, F. Owen, *Life Sci.* **23**, 563 (1978).
13. T. D. Reisine, M. Rossor, E. Spokes, L. L. Iversen, H. I. Yamamura, in *Receptors for Neurotransmitters and Peptide Hormones*, G. Pepeu, M. J. Kuhar, S. J. Enna, Eds. (Raven, New York, 1980).
14. D. R. Burt, I. Creese, S. H. Snyder, *Science* **196**, 326 (1977).
15. P. Seeman, *Pharmacol. Rev.* **32**, 229 (1980).
16. A. V. P. Mackay *et al.*, *Lancet* **1980-II**, 915 (1980).
17. J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972).
18. I. Hanbauer and E. Costa, in *Calcium and Cell Function*, W. Y. Cheung, Ed. (Academic Press, New York, 1980), vol. 1, p. 253.
19. D. Cassel and Z. Selinger, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3307 (1977).
20. M. Rodbell, *Nature (London)* **284**, 17 (1980).
21. D. Cassel, F. Eckstein, M. Larve, Z. Selinger, *J. Biol. Chem.* **254**, 9835 (1979).
22. P. M. Lad, T. B. Nielson, M. S. Preston, M. Rodbell, *ibid.* **255**, 988 (1980).
23. The dissected brain areas, which have been stored at −70°C, were gently homogenized in 10 volumes (weight to volume) of 10 m*M* tris-maleate buffer (*p*H 7.4) containing 2 m*M* EGTA by using glass-teflon homogenizers. Adenylate cyclase activity was determined in a portion of homogenate corresponding to 1 mg of fresh tissue. The incubation mixture contained 82.5 m*M* tris-maleate buffer (*p*H 7.4), 20 m*M* $MgSO_4$, 1 m*M* isobutylmethylxanthine, 0.5 m*M* EGTA, 0.05 m*M* GTP, and 5 m*M* adenosine triphosphate in a final volume of 500 μl. The incubation was carried out in the presence and absence of four different concentrations of NaF (1, 2.5, 5, and 10 m*M*) or guanylimidodiphosphate (5×10^{-6}, 10^{-5}, and 5×10^{-4}*M*) at 30°C for 5 minutes. The reaction was stopped by heating the samples at 90°C for 5 minutes. The amount of cyclic AMP formed was measured by radioimmunoassay (New England Nuclear).
24. J. E. Kleinman *et al.*, *Catecholamines: Basic and Clinical Frontiers*, E. Usdin, I. J. Kopin, J. D. Barchas, Eds. (Pergamon, New York, 1979), p. 1845.
25. R. S. Spitzer, J. Endicott, E. Robins, *Am. J. Psychiatry* **132**, 1187 (1975).
26. J. C. Stoof and J. W. Kebabian, *Nature (London)* **294**, 360 (1981).
27. M. Memo, W. Lovenberg, I. Hanbauer, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4456 (1982).
28. T. E. Cote *et al.*, *Endocrinology* **110**, 812 (1982).
29. A. J. Cross, T. J. Crow, F. Owen, *Prog. Neuropsychopharmacol.* **4**, 147 (1980).
30. We thank E. Costa and W. Lovenberg for scientific advice and for revising the manuscript. This work was supported in part by a grant from the Scottish Rite Schizophrenia Research Program and by NIH grant MH/NS 31862 to the Brain Bank, Harvard Medical School, McLean Hospital.

28 April 1983

Modulation of the Metastatic Capability in B16 Melanoma by Cell Shape

Abstract. *The lung colonization of B16-F1 cells grown in flat and spherical configurations was studied. Cells cultivated in vitro as spheroids on a nonadhesive substrate expressed in a reversible fashion a marked increase in their propensity to establish metastases. The altered metastatic capability was accompanied by a reversible reduction in the accessibility of cell surface proteins to external iodination and by a dramatic decrease in the synthesis of vimentin.*

Metastasis is an active sequential process whereby the cells from the primary growth site invade the intravascular compartment and may attain once again an extravascular position at the target organ, where they proliferate to become metastases (*1*). Using metastatic cell variants exhibiting low, high, or moderate metastatic properties, we found in various tumor systems that highly metastatic cell variants differ from their mildly metastatic counterparts in their pattern of adhesion to the solid substrate as well as in their ability to establish cognitive interactions (*2, 3*). Since the hematogenous spread of tumor cells involves marked alterations in cell shape and morphology, we addressed the possible interrelation between cell shape–responsive growth control and the metastatic phenotype of tumor cells (*3*). The rationale for this approach stems from recent studies suggesting that the loss of cell shape–dependent growth control is a central feature in cell malignancy (*4*). In this study we attempted to determine whether the proliferation of cells under conditions of controlled cell shape may alter the metastatic phenotype of a specific cell line. We utilized the recently developed technology of preparing nonadhesive substrates by coating the culture plates with nontoxic transparent films of poly(2-hydroxyethylmethacrylate) [poly(HEMA)], which allows the control of cell shape (*5*). Growth under these conditions was used as a model system to mimic the different growth patterns obtained in vivo in the extravascular and intravascular compartments. We found that the growth of B16-F1 melanoma cells in vitro in a spherical configuration induced a marked increase in the metastatic capability of the cells in vivo and a faster spreading on tissue culture dishes, as compared to growth as a monolayer in a flat configuration. This altered biological behavior of the cells was accompanied by alterations in the accessibility of the cell surface proteins to external iodination and a dramatic decrease in vimentin biosynthesis. These alterations were reversible on reattachment and spreading, suggesting a central role for cell shape in the modulation of the metastatic capability.

B16-F1 melanoma cells from semiconfluent monolayers were harvested from cultures in their exponential growth phase with 2 m*M* EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS), *p*H 7.2. The cells were then washed and resuspended in PBS. Single-cell suspensions were seeded on control plates or on plates coated with 0.12 percent poly(HEMA) at a density of 10^5 cells per 60-mm plate. At various intervals thereafter the cells were harvested by adding EDTA (10 m*M*) to the growth medium for 5 minutes at 37°C. Gentle

pipetting was sufficient to detach the cells from the monolayer cultures and to separate the cell aggregates formed when the cells were grown on the nonadhesive, poly(HEMA)-coated substrate. After washing with PBS, the cells were examined under a phase microscope to ensure that the cell suspensions were composed of single cells, and cell number was determined with a Coulter counter (model ZBI). The growth curves reveal that the doubling time for B16-F1 melanoma cells cultivated on plastic was 16 hours, while cells cultivated on poly(HEMA)-coated substrates had a doubling time of 24 hours. This difference in doubling time reflects the lower rate of DNA synthesis that we found in spherical B16-F1 cells (*3*).

Figure 1A shows the characteristic bipolar morphology of B16-F1 cells grown on tissue culture plastic. A very different cell morphology was obtained when the cells were cultured on poly(HEMA)-coated plates (Fig. 1, B to D). At 24 hours after seeding (Fig. 1B), single cells could still be observed, but the majority of the cells formed small aggregates. After long periods of cultivation on poly(HEMA)-coated plates [72 hours (Fig. 1C) and 120 hours (Fig. 1D)], there was an increase in the formation of three-dimensional cell aggregates with irregular configurations. Single-cell suspensions were prepared after 120 hours of suspension culture and the cells were replated on control plastic plates. The disaggregated cells adhered to the substrate (Fig. 1E) and, after 24 hours, acquired a flattened morphology with extended cytoplasmic processes.

To test whether growth in vitro under conditions of flat or spherical morphology can alter the metastatic properties of the B16-F1 melanoma cells, single-cell suspensions were prepared from the cultures represented in Fig. 1, A to E, and 5 $\times 10^4$ cells in 0.2 ml of PBS were injected intravenously into C57BL/6 syngeneic recipients. The mice were necropsied 21 days after the injection. Representative lungs from each group of ten mice are shown adjacent to the cultures from which the cells were harvested (Fig. 1, A′ to E′). Tumor nodules in the lungs produced by the injection of B16-F1 cells harvested from monolayers formed discrete lesions in the lung parenchyma (Fig. 1A′). Far more lung colonies developed from injected B16-F1 cells grown in the spherical configuration for 1 day (Fig. 1B′), 3 days (Fig. 1C′), or 5 days (Fig. 1D′) (Table 1), with the colonies varying in size and usually involving most of the lung. Replating the cells after 5 days of suspension culture on control plastic for 24 to 48 hours resulted in reversion to the regular metastatic phenotype displayed by the B16-F1 cells (Fig. 1E′). These results imply that the cell shape–related alterations in metastatic capability is not due to a selection process, but rather to a reversible modulation. The significant increase in the number of lung tumors that developed after injecting cells previously grown in a spherical configuration was accompanied by an increase in the incidence of extrapulmonary metastases (Table 1).

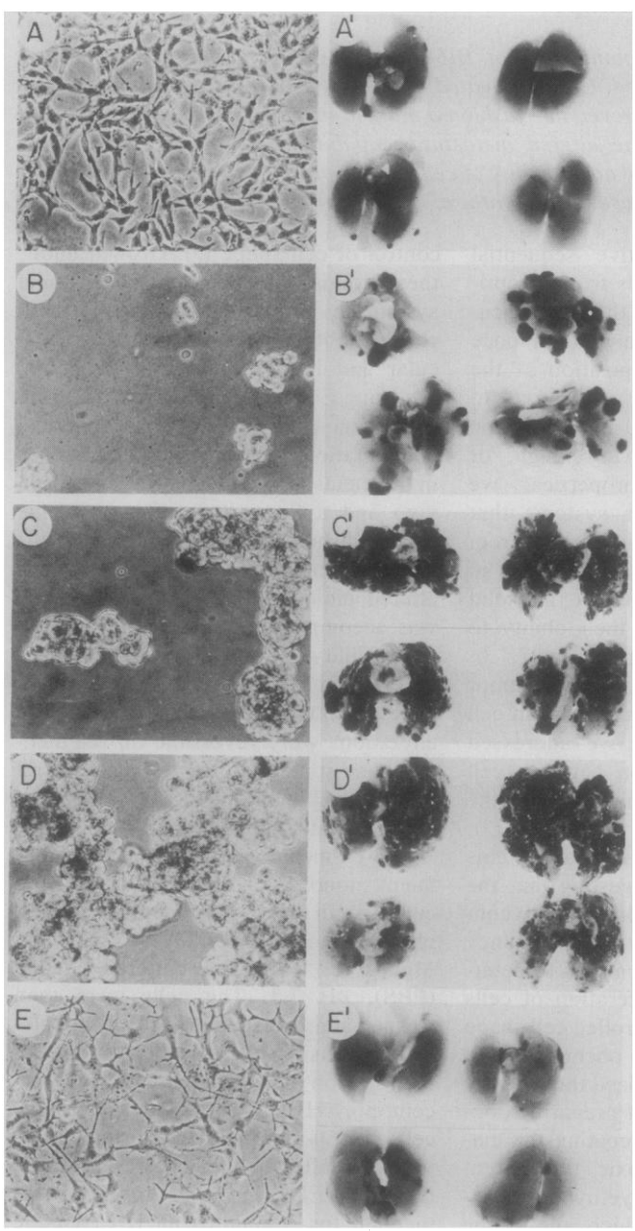

Fig. 1. (A to E) In vitro growth pattern of B16-F1 melanoma cells on plastic (A), on poly(HEMA)-coated plastic for 24 hours (B), 72 hours (C), and 120 hours (D), and on plastic 24 hours after a 120-hour suspension culture (E). (A′ to E′) Gross appearance of lungs from mice given intravenous injections of 5 $\times 10^4$ cells harvested from the corresponding cell cultures. The lungs were excised 3 weeks after inoculation of the tumor cells for determination of relative lung colonization capabilities induced by the various culture conditions.

Since the interaction of the metastasizing cells with the environment and other cells is mediated to a considerable extent by cell surface components, we next examined the pattern of iodinatable surface components obtained after growing the cells in different configurations. Single-cell suspensions prepared by EDTA detachment of cells grown as monolayers on plastic, in a spherical configuration on poly(HEMA)-coated plates for 3 days, or after 20 hours of replating on control plastic were externally iodinated with ^{125}I and crude membrane preparations were analyzed on sodium dodecyl sulfate–polyacrylamide gels. Autoradiography revealed a marked reduction in the availability of cell surface proteins for labeling with ^{125}I in membrane preparations from cells grown in suspension in a spherical configuration (Fig. 2B), as compared to cells grown as a monolayer in a flat configuration (Fig. 2A). The acquisition of a flattened morphology after replating the spherical cells for 20 hours resulted in an increase in the accessibility of the exposed cell surface proteins for iodination (Fig. 2C). In addition, replating EDTA-dispersed cells

from monolayer cultures or from 3-day suspension cultures for 2.5 hours on glass cover slips resulted in adherence of single cells to the substrates, but the replated spherical cells displayed a much faster rate of spreading on the substrate as compared to freshly detached monolayer cells.

These observations, together with the suggestion of a continuity between the cell surface components and the cytoskeleton (*6*), raised the question as to whether there is a difference in the rate of synthesis of intracellular proteins involved in determining cell shape. Cultures of monolayer cells (Fig. 2G) or of cells suspended for 1 (Fig. 2D), 3 (Fig. 2E), or 5 days (Fig. 2F) and replated for 24 hours after 5 days of suspension (Fig. 2H), were incubated for 4 hours with [^{35}S]methionine (40 μCi/ml). The proteins were analyzed by two-dimensional gel electrophoresis. Growth of the cells in a round configuration gradually but extensively down-regulated the synthesis of vimentin, a major cytoskeletal protein, during suspension culture (Fig. 2, D and F). Replating of cells (Fig. 2H) on plastic followed by cell spreading induced recovery of vimentin synthesis to the monolayer rate (Fig. 2G).

There are very few if any in vitro parameters correlated with the metastatic capability of tumor cells (*1*). While studying B16 melanoma variants exhibiting different metastatic potentials, investigators found a correlation between the tendency of the cells to undergo homotypic aggregation in vitro and their ability to produce metastases in vivo (*1*, *2*). Therefore, we analyzed the propensity of single-cell suspensions grown under various configurations to aggregate in vitro. One-milliliter suspensions of single cells (10^6 cells per milliliter) prepared from monolayers or from cells grown for 3 days in suspension were slowly rotated at 37°C in medium in the presence or absence (control) of 10 percent fetal bovine serum. Twenty-one and 47 percent of cells previously grown in monolayers formed aggregates of more than eight cells after 30 and 60 minutes, respectively, while 47 and 62 percent of the cells cultured on poly(HEMA)-coated plates were found in aggregates after 30 and 60 minutes of rotation, respectively. (The two control cell suspensions without serum did not aggregate.) Thus, the ability of B16 melanoma cells to form homotypic aggregates appears to be correlated with the expression of the metastatic phenotype.

The phenomenon of shifts in tumor cell phenotype induced by signals from the environment was recently reviewed (*7*). It was postulated that signals from the environment could regulate the expression of various genetic programs, which in turn could activate or repress various cellular activities that might affect the metastatic properties of the cells. It is now well established that cell shape plays a central role in regulating cell growth (*5*) and is possibly correlated with a variety of growth-related phenomena, such as macromolecular metabolism (*8*), mitogenic stimulation (*9*), differentiation (*10*), and tumorigenicity (*11*). Furthermore, the conversion of murine sarcoma from solid tumors to ascites form (cell suspensions in the peritoneum) was found to be associated with an increased capacity to metastasize to the lung (*12*). The complexity of the metastatic process is most probably the reflection of a delicate balance between multiple gene products. Therefore, the growth of cells in a spherical configuration might have altered this balance and probably endowed some of the formerly noninvasive B16-F1 cells with cellular properties that enabled them to establish metastases. This study suggests that the growth pattern of cells in vivo might augment the overall expression of the metastatic capability of cells. Finally, investigating the reversible modulation of the metastatic phenotype by controlling cell shape might enable the detection of initial cellular changes responsible for the progression from a slightly to a highly metastatic cell variant.

Table 1. Lung nodule formation and tumor distribution in C57BL/6 mice inoculated intravenously with 5×10^4 B16-F1 cells. Single-cell suspensions were maintained in PBS at 4°C prior to injection into the tail vein.

Cell culture condition	Median number of lung nodules (range)	Extrapulmonary tumors	
		Incidence/ animal	Site and number
Control plastic	10 (1 to 25)	0/10	
24 hours on poly(HEMA)-coated plastic	28 (7 to > 200)	4/9	1 omentum, 3 visceral lymph node, 3 thymus
72 hours on poly(HEMA)-coated plastic	> 200 (> 200)	5/10	1 heart, 5 visceral lymph node, 1 ovary
120 hours on poly(HEMA)-coated plastic	> 200 (45 to > 200)	3/10	1 thymus, 3 visceral lymph node
120 hours on poly(HEMA)-coated plastic, 24 hours on control plastic	10 (0 to 29)	0/8	

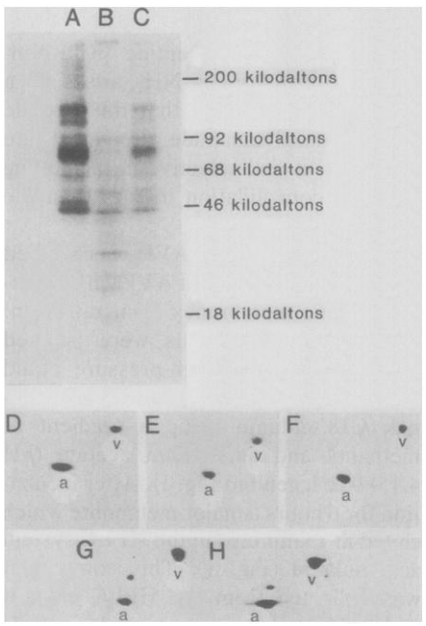

Fig. 2. Single-cell suspensions prepared from B16 melanoma cells grown in monolayer (A), after 3 days in suspension culture (B), or after replating for 24 hours (C) and labeled with $Na^{125}I$ using Iodogene (Pierce Chemical). A soluble fraction was prepared with 0.5 percent deoxycholate and 1 percent Tween 40, and equal amounts of trichloroacetate-precipitable radioactive materials were analyzed on a 7 to 17 percent acrylamide gradient sodium dodecyl sulfate gel. B16 melanoma cells grown in suspension for 1 day (D), 3 days (E), or 5 days (F), replated for 24 hours after 5 days in suspension (H), or grown as a monolayer (G) were labeled for 4 hours with [^{35}S]methionine (40 μCi/ml) and equal amounts of trichloroacetate-precipitable proteins were analyzed by two-dimensional gel electrophoresis (*13*). Abbreviations: *a*, actin; *v*, vimentin.

Note added in proof: We recently obtained a similar (over 100-fold) increase in metastatic capability by growing cells for 3 days in a spherical configuration in two different tumor systems: U.V. 2237-d-15 (fibrosarcoma) and K1735-d-D (melanoma).

AVRAHAM RAZ
Department of Cell Biology, Weizmann Institute of Science, Rehovot, 76100 Israel

AVRI BEN-ZE'EV
Department of Genetics, Weizmann Institute of Science

References and Notes

1. I. J. Fidler, D. M. Gerstein, I. R. Hart, *Adv. Cancer Res.* **28**, 149 (1978).
2. A. Raz, C. Bucana, W. McLellan, I. J. Fidler, *Nature (London)* **284**, 363 (1980); A. Raz and R. Lotan, *Cancer Res.* **41**, 3642 (1981); R. Lotan and A. Raz, *ibid.* **43**, 2088 (1983); A. Raz and B. Geiger, *ibid.* **42**, 5183 (1982).
3. A. Ben-Ze'ev and A. Raz, *Cell* **21**, 365 (1981); A.

Raz and A. Ben-Ze'ev, *Int. J. Cancer* **29**, 711 (1982).
4. J. Folkman and H. P. Greenspan, *Biochim. Biophys. Acta* **417**, 211 (1975); S. C. Wittelsberger *et al.*, *Cell* **24**, 859 (1981).
5. J. Folkman and A. Moscona, *Nature (London)* **273**, 345 (1978).
6. I. Yahara and G. M. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1579 (1975); A. Ben-Ze'ev, A. Duerr, F. Solomon, S. Penman, *Cell* **17**, 859 (1979).
7. V. Schirrmacher, *Immunobiology* **157**, 89 (1980); L. Weiss, *Am. J. Pathol.* **97**, 60 (1978).
8. A. Ben-Ze'ev, S. R. Farmer, S. Penman, *Cell* **21**, 365 (1980).
9. D. Gospodarowicz, G. Greenburg, C. R. Birdwell, *Cancer Res.* **30**, 4155 (1978).
10. P. D. Benya and J. D. Shaffer, *Cell* **30**, 215 (1982).
11. D. Brouty-Boye, R. W. Tucker, J. Folkman, *Int. J. Cancer* **26**, 501 (1980).
12. E. Klein, *Exp. Cell. Res.* **8**, 188 (1955).
13. P. H. O'Farrell, *J. Biol. Chem.* **250**, 4007 (1975).
14. We thank M. Feldman and Y. Aloni for their careful review of the manuscript, R. Karkash and T. Kreizman for technical assistance, and M. Baer and P. Rubinstein for preparation of the manuscript. Supported in part by grants from the Israel Cancer Association (A.R.) and the United States–Israel Binational Foundation (A.B-Z.). A.R. is the incumbent of the Sophie M. T. and Richard S. Richards Career Development Chair in Cancer Research in perpetuity. Avri Ben-Ze'ev is the incumbent of the Ruth and Leonard Simon Career Development Chair in perpetuity.

2 February 1983; revised 11 May 1983

A Major Metabolite of Arginine Vasopressin in the Brain Is a Highly Potent Neuropeptide

Abstract. *A peptide that accumulated as the major product during the proteolysis of arginine vasopressin by rat brain synaptic membranes was isolated and its structure was shown to be the hexapeptide pGlu-Asn-Cys(Cys)-Pro-Arg-Gly-NH_2. When administered intracerebroventricularly in extremely low doses, this vasopressin fragment and its desglycinamide derivative facilitated memory consolidation in a passive avoidance situation. These vasopressin metabolites, which are devoid of pressor activity, constitute highly potent neuropeptides with selective effects on memory and related processes; they are activated via proteolytic processing of vasopressin by brain peptidases.*

The nonapeptide arginine vasopressin (AVP) regulates peripheral as well as central functions. The regulatory role of AVP in the central nervous system is evident in the influence of AVP on learning and memory, brain development, cardiovascular- and thermoregulation, development of tolerance, physical dependence on opiates and ethanol, and drug-seeking behavior (1). Studies on animal behavior and clinical trials point to a memory-improving influence of AVP. In experimental animals AVP improves learning, increases resistance to extinction of active avoidance behavior, facilitates passive avoidance behavior, and protects against retrograde amnesia (2). Several reports describe that in clinical trials AVP or derived peptides improve the performance of patients with certain memory disorders and increase cognitive responses of unimpaired subjects (3).

Structure-activity studies using synthetic AVP fragments have shown that the peripheral and central activities of AVP can be dissociated and that different short sequences derived from the AVP structure affect different aspects of memory (4). Based on these findings the existence of vasopressin fragments with more selective or potent activities has been presumed (4). We have approached the identification of such active AVP fragments by studying the conversion of AVP by proteolytic enzymes in the brain. We report here that during proteolysis of AVP the peptide pGlu-Asn-Cys(Cys)-Pro-Arg-Gly-NH_2 arises as a major metabolite and that this peptide and its desglycinamide derivative are more potent and selective in facilitating memory consolidation in rats than the parent molecule.

The metabolism of AVP was studied by in vitro incubation of AVP with isolated synaptic membranes from rat brain. Products of proteolysis were isolated by reversed-phase high-pressure liquid chromatography (HPLC) on a μBondapak C18 column using a gradient of methanol and ammonium acetate (*p*H 4.15) (see legend to Fig. 1). After incubation for 6 hours a major metabolite which eluted at 15 minutes in the HPLC system accumulated (Fig. 1). This component was collected from the HPLC effluent and subjected to amino acid analysis after performic acid oxidation (5) and determination of free NH_2-terminal groups (6). Analyses demonstrated that the fraction contained a single peptide. The amino acid composition of the isolated peptide was Cys(O_3H), 2.0; Asp, 1.0; Glu, 0.9; Arg, 1.0; and Gly, 1.0 (7). The amounts of Tyr and Phe were below the detection limit. Pro was not detected by this amino acid analysis technique (5). In the end-group analysis monodansyl-Cyt (dansyl-Cys-OH H-Cys-OH) was detected. After treatment of the isolated peptide with pyroglutamate-aminopeptidase (E.C. 3.4.11.8) according to procedures described in (8) dansyl-Asp was detected, demonstrating that the peptide contained pGlu at its NH_2-terminus. The analyses showed that the isolated peptide was the COOH-terminal hexapeptide of AVP having an intact disulfide bridge. The structure of this peptide, which we refer to as [pGlu^4, Cyt^6]AVP-(4–9), is

H-Cys-OH
|
pGlu-Asn-Cys-Pro-Arg-Gly-NH_2

This peptide and its des-Gly-NH_2 derivative [pGlu^4, Cyt^6]AVP-(4–8) were synthesized by the classical approach of fragment condensation. Synthetic and isolated [pGlu^4, Cyt^6]AVP-(4–9) were found to coelute in the reversed-phase HPLC system described in Fig. 1 and in paired-ion HPLC using heptanesulfonic acid as the ion-pairing reagent (9).

The activity of peptides on memory processes was tested in a step-through passive avoidance situation using a single learning trial in male Wistar rats (10). The apparatus consisted of an illuminated, elevated platform attached to a dark compartment. The animals, male Wistar rats weighing 180 to 200 g, were habituated to the dark compartment and were subsequently placed on the illuminated platform to enter the dark. On the following day three more trials were given. At the end of the third trial, upon entering the dark compartment animals received an unescapable foot shock (0.25 mA for 2 seconds) through the grid floor. Immediately after this learning trial animals were treated with 1 μl of peptide in saline or saline alone by intracerebroventricular injection through a stainless steel cannula. The cannula had been placed in the right lateral ventricle at least 5 days before the behavioral experiments started. The influence of substances on the storage of the aversive experience (memory consolidation) was assessed by testing the retention of the passive avoidance response 24 or 48 hours after the learning trial. Retention of passive avoidance behavior was measured by the latency to reenter the dark compartment up to a maximum of 300 seconds after placing the animals on the illuminated platform.

Arginine vasopressin caused a significant facilitation of passive avoidance behavior in doses of 100 pg and higher (Table 1). The activity of [pGlu^4, Cyt^6]-AVP-(4–9) was tested in a dose range of 0.01 to 1000 pg. The peptide displayed an inverted U–shaped dose-response relationship: in extremely low doses, 0.03 and 0.10 pg, the peptide facilitated passive avoidance behavior; the lowest effective dose at the 24-hour latency was