Inhibition of Forskolin-induced Neurite Outgrowth and Protein Phosphorylation by a Newly Synthesized Selective Inhibitor of Cyclic AMP-dependent Protein Kinase, N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D Pheochromocytoma Cells*

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A newly synthesized isoquinolinesulfonamide, H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide), was shown to have a potent and selective inhibitory action against cyclic AMP-dependent protein kinase (protein kinase A), with an inhibition constant of 0.048 ± 0.008 μM. H-89 exhibited weak inhibitory action against other kinases and K values of the compounds for these kinases, including cGMP-dependent protein kinase (protein kinase G), Ca2+/phospholipid-dependent protein kinase (protein kinase C), caspase kinase I and II, myosin light chain kinase, and Ca2+/calmodulin-dependent protein kinase II were 0.48 ± 0.13, 31.7 ± 15.9, 38.3 ± 6.0, 136.7 ± 17.0, 28.3 ± 17.5, and 29.7 ± 8.1 μM, respectively. Kinetic analysis indicated that H-89 inhibits protein kinase A, in competitive fashion against ATP. To examine the role of protein kinase A in neurite outgrowth of PC12 cells, H-89 was applied along with nerve growth factor (NGF), forskolin, or dibutyryl cAMP. Pretreatment with H-89 led to a dose-dependent inhibition of the forskolin-induced protein phosphorylation, with no decrease in intracellular cyclic AMP levels in PC12D cells, and the NGF-induced protein phosphorylation was not inhibited. H-89 also significantly inhibited the forskolin-induced neurite outgrowth from PC12D cells. This inhibition also occurred when H-89 was added before the addition of dibutyryl cAMP. Pretreatment of PC12D cells with H-89 (30 μM) inhibited significantly cAMP-dependent histone IIb phosphorylation activity in cell lysates but did not affect other protein phosphorylation activity such as cGMP-dependent histone IIb phosphorylation activity, Ca2+/phospholipid-dependent histone IIIb phosphorylation activity, Ca2+/calmodulin-dependent myosin light chain phosphorylation activity, and α-casein phosphorylation activity. However, this protein kinase A inhibitor did not inhibit the NGF-induced neurite outgrowth from PC12D cells. Thus, the forskolin- and dibutyryl cAMP-induced neurite outgrowth is apparently mediated by protein kinase A while the NGF-induced neurite outgrowth is mediated by a protein kinase A-independent pathway.

Since the discovery of cAMP-dependent protein kinase (protein kinase A) in rabbit skeletal muscle by Krebs and colleagues (Walsh et al., 1968), the mechanisms of action of a number of additional extracellular signals have been found to involve regulation of the formation of cAMP and thereby control of its target enzyme (Greengard, 1978).

Extension of the neuronal process is fundamental to establishment of the intricate network of the nervous system. The rat PC12 pheochromocytoma cell line is a pertinent model system for the study of neuronal differentiation (Greene and Tischler, 1976). Nerve growth factor (NGF) is a well characterized polypeptide (Levi-Montalcini and Angeletti, 1968) which promotes a variety of responses in target cells (Greene and Tischler, 1976; Greene and Shooter, 1980). PC12 responds to NGF with neurite outgrowth (Greene and Tischler, 1976), increases in the rate of acetylcholine synthesis (Greene and Tischler, 1976; Greene and Rein, 1977), alterations in the surface morphology (Cumnolly et al., 1979), activation of adenylate cyclase, and generation of intracellular cAMP (Schubert et al., 1978). Permeable analogues of cAMP or a cAMP-enhancing reagent can elicit responses which mimic the action of NGF in PC12 cells (Greene and McGuire, 1978; Rieger et al., 1990; Hagegous and Patrick, 1980; Greene et al., 1984). The role of cAMP in the action of NGF remains unclear and whether or not the enhancement of intracellular cAMP-induced function in PC12 cells is mediated by protein kinase A is also unknown. A subline of PC12 cells, PC12D cells, extends neurites very quickly in response not only to NGF but also to cAMP-enhancing agents (Katoh-Semba et al., 1987). To explore further the role of protein kinase A on neurite outgrowth, we investigated the effect of H-89, a newly synthesized protein kinase A inhibitor, on forskolin-, dibutyryl cAMP-, and NGF-induced neurite outgrowth from PC12D cells.

The abbreviations used are: NGF, nerve growth factor; H-89, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide; H-88, N-[2-cinnamylaminoethyl]-5-isoquinolinesulfonamide; H-85, N-[2-(N-formyl-p-chlorocinnamylamino)ethyl]-5-isoquinolinesulfonamide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

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EXPERIMENTAL PROCEDURES

Materials—Histone IIa, histone I2b, calf casein, and forskolin were purchased from Sigma. Phosphatidylserine was purchased from Ser-dary Research Laboratory. [γ-32P]ATP and [γ-32P]Phosphorus were obtained from ICN Radiochemicals.

Protein kinase C from rabbit brain was purified as described (Hidaka and Tanaka, 1987), and cAMP-dependent protein kinase catalytic subunit was prepared from bovine heart by the method of Beavo et al. (1974). cGMP-dependent protein kinase was prepared from pig lung by the method of Kuriyama and Goedde (1974). Casein kinase I and II from bovine testes were purified as described (Chijiwa et al., 1989). Ca\(^{2+}\)/calmodulin-dependent protein kinase II was prepared from rat brain by the method of McGuinness et al. (1985). Myosin light chain was prepared from chicken gizzard by the method of Perrie and Perry (1970). Myosin light chain kinase was purified from chicken gizzard by the method of Adelstein and Rhee (1981). Calmodulin was isolated from bovine brain and purified by the procedure described by Yazawa et al. (1986).

Duolbecco’s modified Eagle’s medium containing high glucose was purchased from GIBCO. Fetal calf serum and horse serum were from M.A. Bioproducts. CAMP determination kits were purchased from Yamasa Chemical Corp. H-89 and H-88 were derivatives of H-8 (N-(2-cinnamylaminoethyl)-5-isoquinolinesulfonamide), a cyclic nucleotide-dependent protein kinase inhibitor. Isoquinolinesulfonamide derivatives were synthesized by the method of Hidaka et al. (1984).

Protein was determined by the method of Lowry et al. (1951).

Enzyme Assay and Determination—Protein kinase C, cAMP-dependent protein kinase, cGMP-dependent protein kinase, and myosin light chain kinase activity were measured using the conditions described by Hidaka et al. (1984). Casein kinase I and II activities were measured as described (Chijiwa et al. 1989). Ca\(^{2+}\)/calmodulin-dependent protein kinase II activity was measured by the method described by McGuinness et al. (1985). Kinase activities were assayed at 30 °C for 2 to 5 min by measuring the transfer of \(^{32}\)P from [\(^{-32}\)P]ATP to substrates. The reaction was terminated by adding 1 ml of 20% trichloroacetic acid, and the residue sample solution was used for determination. Protein was estimated according to the method of Lowry and colleagues (1951).

Cell Culture— Cultures of PC12D cells were expanded in 100-mm tissue culture plastic dishes. The medium used was Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum, 10% horse serum, 5 μg/ml insulin, 5 μg/ml transferrin, 10\(^{-4}\) M putrescine, 12 × 10\(^{-8}\) M progesterone, and 3 × 10\(^{-8}\) M sodium selenite. We replated the subconfluent cells on 6-well tissue culture plastic plates at a density of 4 × 10\(^5\) cells/well for analysis of neurite outgrowth and at a density of 4 × 10\(^5\) cells/well for analysis of protein phosphorylation and CAMP accumulation.

Determination of Intracellular Levels of cAMP in PC12D Cells—Levels of intracellular cAMP were determined with the use of protocol and kit purchased from Yamasa. After 48 h in culture, PC12D cells were cultured in test medium containing 30 μM H-89 for 1 h and then exposed to fresh medium that contained both 10 μM forskolin and 30 μM H-89. Cells were scraped off with a rubber policeman and sonicated in the presence of 0.5 ml of 6% trichloroacetic acid. To extract trichloroacetic acid, 2 ml of petroleum ether was added, the preparation mixed and centrifuged at 3000 rpm for 10 min. After aspiration of the upper layer, the residue sample solution was used for determination.

Protein was estimated according to the method of Lowry et al. (1951).

Morphological Observations—Cells were exposed to 20 μM H-89 for 30 min prior to stimulation with 10 μM forskolin or 50 μg/ml NGF or 1 mM dibutyryl cAMP. Processes with lengths equivalent to two or more diameters of a cell body were scored as neurites. The morphological differentiation of PC12D cells was assayed by determining the number of processes that were outgrowth cells.

Labeling of Cultured Cells with \([\gamma-32P]\)Phosphorus and Protein Phosphorylation—The tissue culture medium was removed and the cell monolayers were incubated for 1 h in phosphate free HEPES Krebo solution (HEPES-KR). The HEPES-KR was then replaced with HEPES-KR supplemented with 50-60 μCi/ml \([\gamma-32P]\)phosphorus with 20-200 μCi/ml H-89, and the tissue cultures were incubated for 1 h in a CO\(_2\) atmosphere. Then HEPES-KR was replaced with Dulbecco’s modified Eagle’s medium which was supplemented with H-89. Labelled cultures were harvested at various time periods in 0.2 ml of sample buffer containing 8 M urea, 10% SDS, 10 mM mercaptoethanol, and Tris-HCl buffer (pH 7.4). The samples were separated by 10% SDS-polyacrylamide gel electrophoresis. The gels were stained with Coomassie Brilliant Blue and placed in x-ray cassettes containing Kodak X-Omat AR film for autoradiography.

RESULTS

Effect of H-89 on Various Protein Kinase Activities in Vitro—The effects of this newly synthesized isoquinolinesulfonamide on cAMP-dependent protein kinase (protein kinase A), cGMP-dependent protein kinase (protein kinase G), Ca\(^{2+}\)/phospholipid-dependent protein kinase, (protein kinase C), myosin light chain kinase, Ca\(^{2+}\)/calmodulin-dependent protein kinase II (Ca\(^{2+}\)/calmodulin kinase II), and casein kinase I and II were investigated. K\(_v\), values for various protein kinases are summarized in Table I. As can be seen, H-88 (N-(2-cinnamylaminoethyl)-5-isoquinolinesulfonamide) exhibited a potent inhibitory action on cyclic nucleotide-dependent protein kinases. H-89, a brominated derivative of H-88, proved to be the most potent and selective cAMP-dependent protein kinase inhibitor among the isoquinolinesulfonamide compounds tested, and the K\(_v\), value for protein kinase A was 0.048 ± 0.008 μM. The K\(_v\), value of this drug for protein kinase G was about 10 times higher (0.48 ± 0.13 μM), suggesting that H-89 was a specific protein kinase A inhibitor (Table I). H-89 was shown to have a more selective inhibition for protein kinase A than for protein kinase G and much weaker effects for other kinases (at least 500-3000-fold less potent). The structures of these protein kinase A inhibitors are shown in Fig. 1. Inhibition patterns of protein kinase A by H-89 and

| Kinase | H-85 | H-89 |
|-------|------|------|
| cAMP-dependent protein kinase | 0.36 ± 0.06 | 0.048 ± 0.008 |
| Protein kinase C | 0.76 ± 0.05 | 0.48 ± 0.13 |
| Myosin light chain kinase | 75.7 ± 9.6 | 31.7 ± 15.9 |
| Ca\(^{2+}\)/calmodulin kinase II | 50.0 ± 8.2 | 28.3 ± 17.5 |
| Casein kinase I | 71.3 ± 11.9 | 29.7 ± 8.1 |
| Casein kinase II | 60.7 ± 12.2 | 38.3 ± 6.0 |

**Table I**

**Effects of H-88 and H-89 on various protein kinase activities in vitro**

Enzyme activities were determined as described under “Experimental Procedures.” 40 μg of histone IIb for cAMP-dependent protein kinase (0.8 μg) and cGMP-dependent protein kinase (0.6 μg), 40 μg of histone IIa for Ca\(^{2+}\)/phospholipid-dependent protein kinase (2.47 μg), 10-20 μg of myosin light chain for myosin light chain kinase (1.4 μg) and for Ca\(^{2+}\)/calmodulin-dependent protein kinase II (0.6 μg) and 120 μg of casein for casein kinase I (2.9 μg) and II (2.5 μg) were used for substrates. ATP was present at a final concentration of 5, 10, 20, and 30 μM for each kinases. Assays were performed at 30 °C for 2-5 min. The values were estimated by Dixon plots. Each value represents the mean ± S.D. of three determinations.

**Fig. 1.** Chemical structures of newly synthesized compounds, H-88 and H-89.
H-89 and H-88 were then analyzed by double reciprocal plots (Fig. 2). H-89 and H-88 inhibited the kinase activity competitively with ATP. To investigate whether these inhibitors could serve as pharmacological tools for examining viable cells, we studied the effects of H-89, on forskolin- and NGF-induced protein phosphorylation in PC12D cells.

**Forskolin-induced Protein Phosphorylation in the Presence or Absence of H-89 in PC12D Cells**—Fig. 3 shows that forskolin (10 μM) induced protein phosphorylation in the PC12D cells. In the absence of forskolin, 32P-radioactive phosphorylated proteins were not detected in PC12D cells. One h before the addition of forskolin, pretreatment of the cells with H-89 (20 or 30 μM) markedly inhibited the forskolin-induced protein phosphorylation in a dose-dependent manner at 60 min after the addition of 10 μM of forskolin (Fig. 3A). These inhibitory effects were recognized even at 8 h after the addition of forskolin (data not shown). The inhibition of NGF (50 ng/ml)-induced protein phosphorylation was not observed in the PC12D cells pretreated with H-89 (Fig. 3B). These results suggest that H-89 is a more useful compound with respect to the selective inhibition of protein kinase A in vivo. The effects of H-89 on forskolin-induced cAMP accumulation in PC12D cells were also examined. The addition of H-89 (30 μM) along with 10 μM forskolin did not affect forskolin-induced enhancement of the cAMP levels in PC12D cells (20 and 40 min after addition of forskolin: H-89, 126.3 ± 9.3 and 166.7 ± 5.1; without H-89, 124 ± 3.2 and 176 ± 9.2 pmol/mg protein, respectively; the cAMP level without forskolin, 28.6 ± 4.2; mean ± S.E., n = 3). When examining the effect of H-89 on adenylate cyclase from bovine brain and cyclic 3',5'-nucleotide phosphodiesterase from rabbit brain, we found that this compound did not inhibit these enzymes up to a dose of 100 μM. Neither H-89 nor forskolin directly influenced activities of cyclic AMP synthesizing (adenylate cyclase) and metabolizing (phosphodiesterase) enzymes in vitro. Thus, H-89 may act directly on the protein kinase A in the PC12D cells.

**Forskolin-, NGF-, and Dibutyryl cAMP-induced Neurite Outgrowth, with or without the Protein Kinase A Inhibitor H-89 from PC12D Cells**—To investigate the role of protein kinase A on differentiation of PC12D cells, the effect of H-89 (20 μM) on forskolin (10 μM)-, NGF (50 ng/ml)-, and dibutyryl cAMP (1 mM)-induced neurite outgrowth at various time points was investigated using phase contrast microscopy. Neurite outgrowth was maximal at 16 h after the addition of forskolin, and this outgrowth was maintained for 48 h (Fig. 4A). When the cells were pretreated with H-89 for 30 min before the addition of forskolin, H-89 (20 μM) significantly inhibited the forskolin-induced neurite outgrowth from 8 to 48 h after the addition of forskolin. We next investigated the
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**FIG. 5.** Photomicrographs of forskolin-induced neurite outgrowth with or without H-89 from PC12D cells. PC12D cells were incubated for 8 h with (B) or without (A) 20 μM H-89 and for 24 h with (D) or without (C) 20 μM H-89 in the presence of 10 μM forskolin.

Effects of H-89 on NGF-induced neurite outgrowth from PC12D cells. Exposure of NGF (50 ng/ml) produced a stimulation of neurite outgrowth in a time-dependent fashion, and the maximal effect was seen at 24 h (Fig. 4B). Pretreatment with H-89 (20 μM) for 30 min before the addition of NGF (50 ng/ml) exerted no inhibitory action on the NGF-induced neurite outgrowth of PC12D cells. We also investigated the effect of H-89 on dibutyryl cAMP-induced neurite outgrowth of the PC12D cells (Table II). After pretreatment with H-89 for 30 min, PC12D cells were exposed for 24 h to culture medium which contained 1 mM dibutyryl cAMP. H-89 inhibited dibutyryl cAMP-induced neurite outgrowth dose-dependently. The finding that H-89 inhibited both forskolin- and dibutyryl cAMP-induced neurite outgrowth in PC12D cells suggests that the inhibitory action of H-89 is not mediated through the inhibition of cAMP synthesis or degradation. It would thus appear that protein kinase A acts as mediator of forskolin- or dibutyryl cAMP-induced neurite outgrowth but not of NGF-induced neurite outgrowth. Figs. 5 and 6 show typical photographs of forskolin-, NGF-, and dibutyryl cAMP-induced neurite outgrowth, with or without H-89.

**Effects of Other Kinase Inhibitors and Negative Control, H-85, on Forskolin-induced Neurite Outgrowth from PC12D Cells**—When the cells were treated with 30 μM ML-9, a specific myosin light chain kinase inhibitor (Saitoh et al., 1987), H-7, a protein kinase C inhibitor (Hidaka et al., 1984), CKI-7, a selective casein kinase I inhibitor (Chijiwa et al., 1989), or KN-62, a specific Ca<sup>2+</sup>/calmodulin kinase II inhibitor (Tokumitsu et al., 1990), none inhibited a forskolin (10 μM)-induced neurite outgrowth of PC12D cells, respectively (data not shown). H-85, N-[2-(N-formyl-p-chlorocinnamylamino)ethyl]-5-isoquinolinesulfonamide, was chosen as a control agent of H-89 and H-88 because H-85, a derivative of H-89, was shown to have similar IC<sub>50</sub> values for the kinases, except for protein kinases A, G, and C. The IC<sub>50</sub> values of this compound for various protein kinases were as follows: >100 μM for protein kinases A, G, and C; 28 μM for myosin light chain kinase; 47 μM for Ca<sup>2+</sup>/calmodulin kinase II; 50 μM for casein kinase I; and >100 μM for casein kinase II. H-85 was not selective in producing inhibition of protein kinase A but did produce a similar inhibition on other kinases; hence this compound can be used as a negative control of H-89. Thirty μM of this compound did not inhibit the forskolin-induced neurite outgrowth of PC12D cells (Table III).

**Effect of H-89 on Various Protein Kinases in Cell Lysates of PC12D Cells**—To investigate the effect of pre-treatment on H-89 on various protein kinase activities in PC12D cells, cell lysates of PC12D cells were used. Forty μl each of all lysates with or without pre-treatment of H-89 (30 μM) were used to measure protein kinases A, G, and C, Ca<sup>2+</sup>/calmodulin-dependent protein kinase, and α-casein phosphorylation activity. Kinase reactions were initiated by addition of 10 μM [γ-<sup>32</sup>P]ATP and incubated for 5 min at 30 °C. Protein kinase A activities of cell lysates in PC12D cells with or without pre-treatment of H-89 (30 μM) were 1.3 ± 0.6 and 6.7 ± 1.6 (picomoles of P/5 min), respectively (Table IV). H-89 significantly inhibited protein kinase A activity in cell lysates of PC12D cells, but H-89 did not affect other kinase activity such as protein kinases G and C, Ca<sup>2+</sup>/calmodulin-dependent protein kinases, and α-casein phosphorylation activity. We also investigated the effect of H-89 on forskolin-activated kinase activity of cell lysate in PC12D cells. Histone IIb phosphorylation activity of cell lysate was measured without cAMP. After pre-treatment of H-89 (30 μM) for 1 h in PC12D cells, forskolin (10 μM) was added and incubated for 30 min, and then the cells were immediately washed two times with cold Krebs-Henseleit buffer. The cells were quick-frozen in liquid nitrogen. Aliquots of dissolved cell lysates were used to investigate histone IIb phosphorylation activity. Phosphorylation activity was measured by incubating at 30 °C for 5 min after addition of [γ-<sup>32</sup>P]ATP. Forskolin (10 μM)-activated histone IIb (1 mg/ml) phosphorylation activities of cell lysates of PC12D cells with or without H-89 (30 μM) were 0.7 ± 0.3 and 6.9 ± 3.5 (picomoles of P/5 min), respectively. These results suggest that H-89 also selectively inhibited protein kinase A activity of PC12D cells.

**DISCUSSION**

There is some discrepancy regarding the effective dose of H-89 between in vitro and in vivo systems. This can be
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Fig. 6. Photomicrographs of NGF- or dibutyryl CAMP-induced neurite outgrowth in the presence or absence of H-89 from PC12D cells. PC12D cells were incubated for 24 h in the presence of 50 ng/ml NGF only (A), in the presence of 50 ng/ml NGF and 20 μM H-89 (B), in the presence of 1 mM dibutyryl CAMP only (C), and in the presence of 1 mM dibutyryl CAMP and 10 μM H-89 (D).

Explained as follows: presumably it is related to the permeability of H-89 in cell membranes and the high concentration of ATP in the initial phase of the experiment, as observed in the case of H-8 (Tash et al., 1986). Results in Table IV support this assumption. Thus, pretreatment of PC12D cells with 30 μM H-89 occurred to selectively inhibit protein kinase A activity without inhibition of other kinase activities in each lysate. Our present results provide the first evidence that forskolin-induced neurite outgrowth is mediated through protein kinase A. No direct evidence regarding the role of protein kinase A on neurite outgrowth of PC12 cells by forskolin has been presented, even though intracellular CAMP levels were reported to be elevated after the treatment of the cells with forskolin. It has been argued whether CAMP mediates the action of NGF on neurite outgrowth. Detailed biochemical mechanisms related to NGF-induced neurite outgrowth have yet to be determined. Some workers suggested that the cAMP level increased after the exposure of NGF in PC12 cells and that CAMP is a second messenger of NGF (Schubert and Whitlock, 1977; Schubert et al., 1978), while several groups reported that NGF has the ability to enhance cAMP levels but that cAMP is not the second messenger of NGF (Gunning et al., 1981; Greene et al., 1979). It was reported that NGF is unable to enhance cAMP levels in PC12 cells (Hatanaka et al., 1978). Our results suggest that even if NGF is able to enhance cAMP levels in PC12D cells, NGF-induced neurite outgrowth is not mediated through protein kinase A, H-89 is one derivative of H-8, a specific inhibitor of cyclic nucleotide-dependent protein kinase (Hidaka et al., 1984). The anti-protein kinase A activity of H-89 is 30 times more potent than that of H-8, whereas the anti-protein kinase G activity is 10 times less potent. These newly synthesized selective protein kinase A inhibitors H-88 and H-89 should serve as useful tools for clarifying the physiological roles of protein kinase A.

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REFERENCES

Adelstein, R. S., and Klee, C. B. (1981) J. Biol. Chem. 256, 7501–7509
Beavo, J. A., Bechtel, P. J., and Krebs, E. G. (1974) *Methods Enzymol.* 38, 299–308
Chijiwa, T., Hagiwara, M., and Hidaka, H. (1989) *J. Biol. Chem.* 264, 4924–4927
Connolly, J. L., Greene, L. A., Viscarello, R. R., and Riley, W. D. (1979) *J. Cell Biol.* 82, 820–827
Greene, L. A., and McGuire, J. C. (1978) *Nature* 276, 191–193
Greene, L. A., and Rein, G. (1977) *Nature* 268, 349–351
Greene, L. A., and Shooter, E. M. (1980) *Annu. Rev. Neurosci.* 3, 355–402
Greene, L. A., and Tsichler, A. S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 2424–2428
Greene, L. A., Burstyn, D. E., McGuire, J. C., and Black, M. M. (1979) *Soc. Neurosci. Symp.* 4, 153–171
Greene, L. A., Seeley, P. J., Rulfsen, A., DiPiazza, M., Howard, A. (1986) *J. Neurochem.* 42, 1798–1734
Greengard, P. (1978) *Science* 199, 146–152
Gunning, P. W., Landreth, G. E., Bothwell, M. A., and Shooter, E. M. (1981) *J. Cell Biol.* 89, 240–245
Halegoua, S., and Patrick, J. (1980) *Cell* 22, 571–581
Hatanaka, H., Otten, U., and Thuenen, H. (1978) *FEBS Lett.* 92, 313–316
Hidaka, H., and Tanaka, T. (1987) *Methods Enzymol.* 139, 570–582
Hidaka, H., Inagaki, M., Kawamoto, S., and Sasaki, Y. (1984) *Biochemistry* 23, 5036–5041
Katoh-Semba, R., Kitajima, S., Yamazaki, Y., and Sano, M. (1987) *J. Neurosci. Res.* 17, 36–44
Kuo, J. F., and Greengard P. (1974) *Methods Enzymol.* 38, 329–350
Levi-Montalcini, R., and Angeletti, P. U. (1968) *Physiol. Rev.* 48, 534–569
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
McGuinness, T. L., Lai, Y., and Greengard, P. (1985) *J. Biol. Chem.* 260, 1696–1704
Perrie, W. T., and Perry, S. V. (1970) *Biochem. J.* 119, 31–38
Rieger, F., Shelanski, M. L., and Greene, L. A. (1980) *Dev. Biol.* 76, 238–243
Saitoh, M., Ishikawa, T., Matsushima, S., Naka, M., and Hidaka, H. (1987) *J. Biol. Chem.* 262, 7796–7801
Schubert, D., and Whitlock, C. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 4055–4058
Schubert, D., LaCorbiere, M., Whitlock, C., and Stallcup, W. (1978) *Nature* 273, 718–723
Tash, J. S., Hidaka, H., and Means, A. R. (1986) *J. Cell Biol.* 103, 649–655
Tokumitsu, H., Chijiwa, T., Hagiwara, M., Mizutani, A., Terasawa, M., and Hidaka, H. (1990) *J. Biol. Chem.* 265, 4315–4320
Walsh, D. A., Perkins, J. P., and Krebs, E. G. (1988) *J. Biol. Chem.* 263, 3763–3765
Yazawa, M., Sakuma, M., and Yagi, K. (1980) *J. Biochem. (Tokyo)* 87, 1313–1320
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