

# Monensin Inhibits the Binding of <sup>3</sup>H-Flunitrazepam to and Reveals the Intracellular Passage of GABA<sub>A</sub>/Benzodiazepine Receptor

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**Abstract** Effects of monensin were examined on the intracellular processing of the GABA<sub>A</sub>/benzodiazepine receptor (GABA<sub>A</sub>/BZDR) in neuron cultures derived from embryonic chicken brain, using <sup>3</sup>H-flunitrazepam as the probe for the benzodiazepine modulator site on the receptor. Incubation of cultures with 0.1 or 1 μM monensin for 3 h blocked the binding of <sup>3</sup>H-flunitrazepam by about 18%. Loss of ligand binding was due to a reduction in the number of binding sites, with no significant changes in receptor affinity. The general cellular protein synthesis and glycosylation in the cells were inhibited by 26% and 56%, respectively, in the presence of 1 μM monensin, as detected by assaying the incorporation of <sup>3</sup>H-leucine and <sup>3</sup>H-galactose. In contrast, an increase was observed for mannose incorporation by the cultures in the presence of the drug. Moreover, the results from *in situ* trypsinization of the cultures following monensin treatment showed that monensin did not alter the distribution of intracellular and surface receptors. The data suggest that monensin induces the down-regulation of GABA<sub>A</sub>/BZDR by generating abnormal glycosylation of the receptor and interrupting its transport within the Golgi apparatus, as well as from the Golgi apparatus to the intracellular pool and cell membrane. The galactosylation of receptor proteins may be important for the maturation of the receptor. © 1992 Wiley-Liss, Inc.

**Key words:** neuron culture, reversible binding, protein synthesis, glycosylation, Golgi apparatus

The major inhibitory neurotransmitter receptor, GABA<sub>A</sub>/BZD receptor, in the vertebrate brain is a ligand-gated ion channel and a heterooligomer composed of several different subunits (Barnard et al., 1987; for reviews see Olsen and Tobin, 1990; Stephenson, 1991). The receptor is involved in mediating neuronal excitability through combining with GABA to produce an increase in chloride conductance. The function of the receptor can be modulated allosterically by the binding of some therapeutically important classes of drugs, notably the BZDs and the barbiturates, to distinct sites within the receptor (reviewed by Olsen and Venter, 1986; Costa, 1991). It has been shown that the receptor is a glycoprotein and the subunits are all glycosylated; the potential asparagine-glycosylation sites have been proposed for the subunit polypeptide from molecular cloning studies (Sweetnam et al., 1987; Olsen and Tobin, 1990). Although recent advancement on the understanding of

the molecular biology of the receptor has revealed its structural complexity and heterogeneity, the mechanisms responsible for the intracellular processing, such as assembly, maturation, and turnover of the receptor, remain to be elucidated (Molher et al., 1990; Vicini, 1991 for reviews).

Previous studies have shown that one-fifth of the GABA<sub>A</sub>/BZDRs in neurons are intracellular and a newly synthesized pool of receptors passes through this intracellular pool to the cell surface. In addition, this intracellular pool displays identical kinetics of degradation to that of the total receptor (Borden et al., 1984; Czajkowski and Farb, 1986, 1989b; Czajkowski et al., 1989a). In this report, monensin, a Na<sup>+</sup>/H<sup>+</sup> ionophore has been employed to study the intracellular transport of the receptor, since this reagent raises the intracellular pH, causing changes in cellular structure and function (Maxfield, 1982; for review, see Mollenhauer et al., 1990). It is known that monensin interferes with secretion and intracellular transport of a variety of molecules. The principal site of action of monensin is in the Golgi complex; thus, impaired glycosylation of newly synthesized proteins has been

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observed (Tartakoff and Vassalli, 1979; Hamerschlag et al., 1982; Jacobs et al., 1983; Kuhn et al., 1986). Moreover, the post-translational modification and glycosylation have been shown to be associated with normal expression and transport of some membrane glycoproteins, including the nicotinic acetylcholine receptor (Blount and Merlie, 1990; Jarvis et al., 1990).

In this report, the effect of monensin on the binding of <sup>3</sup>H-flunitrazepam (FNZ) to dissociated neuron culture was explored by monitoring the incorporation of amino acid and sugars by the culture. Furthermore, *in situ* trypsinization of monensin-treated cells followed by binding assay was carried out to differentiate the cell surface and intracellular receptors. The results indicated that a decrease in the number of <sup>3</sup>H-FNZ binding sites induced by monensin was due partially to the depression of amino acid and galactose incorporation, and the normal distribution of cell surface and intracellular <sup>3</sup>H-FNZ binding was not changed by monensin.

## MATERIALS AND METHODS

Dissociated neuron cultures were prepared from using the brains of seven-day embryonic chicken as described previously (Czajkowski and Farb, 1986). The cultures were maintained for one week before the following experiments were performed. The cultures were treated with monensin dissolved in 95% ethanol at 0.1, 1, or 10  $\mu$ M for 3 h. The cells were then washed, scraped from the plates, homogenized with a glass homogenizer in PBS and centrifuged. The pellets were rehomogenized. Reversible binding of <sup>3</sup>H-FNZ (NEN, specific activity: 87 Ci/mmol) was carried out by incubating aliquots of culture homogenate with 2–25 nM <sup>3</sup>H-FNZ, according to the procedures described before (Borden and Farb, 1988).

For *in situ* trypsinization experiments, after a 3-h incubation with monensin (1  $\mu$ M) or cycloheximide (40  $\mu$ g/ml of culture medium) the culture received trypsin (0.5 mg/ml) in HEPES buffer for 90 min in the presence of the drugs at 37°C. After trypsinization, the cells were collected and subjected to reversible binding (Czajkowski and Farb, 1986). Non-specific binding was determined in the presence of 1 mM flunitrazepam in all cases and was subtracted from total binding to yield specific binding. The latter was normalized to cpm or dpm per 100  $\mu$ g of

membrane proteins. Parallel incubation and binding of control cultures were performed.

Amino acid or sugar incorporation by the cultures was performed by incubating the cultures with <sup>3</sup>H-leucine (specific activity: 153 Ci/mmol) at 0.4  $\mu$ Ci/ml, <sup>3</sup>H-mannose (13.9 Ci/mmol) at 10  $\mu$ Ci/ml or <sup>3</sup>H-galactose (25.5 Ci/mmol) at 5  $\mu$ Ci/ml in the presence or absence of monensin for 3 h. They were then washed 4 times with PBS and incubated with 1 ml SDS/NP-40/urea (0.02%/2%/8 M) per dish for 1 h on a shaker. Ten percent trichloroacetic acid was used to precipitate the proteins in the sample, followed by the addition of 1 N NaOH. TCA-precipitable proteins were collected by filtering through glass fiber filter (Whatman GF/B); radioactivity associated with the filter was measured after adding liquiscint (National Diagnostics). For <sup>3</sup>H-mannose and <sup>3</sup>H-galactose incorporation experiments, the culture medium was replaced by glucose-free medium for 30 min before the addition of <sup>3</sup>H-mannose or galactose.

All determinations were done in triplicate. The results are presented as the means  $\pm$  standard deviations. Scatchard plots were analyzed by using the ligand program (Munson and Rodbard, 1980). Significance was analyzed using two-tail Student's *t*-test.

## RESULTS

Following exposure of cultures to 0.1, 1, or 10  $\mu$ M monensin for 3 h, the binding of <sup>3</sup>H-FNZ was blocked by about 18%, 18%, and 40% at the three concentrations, respectively (Fig. 1; Table I). The blockade was reversible for 0.1 and 1  $\mu$ M monensin, since the binding was restored to

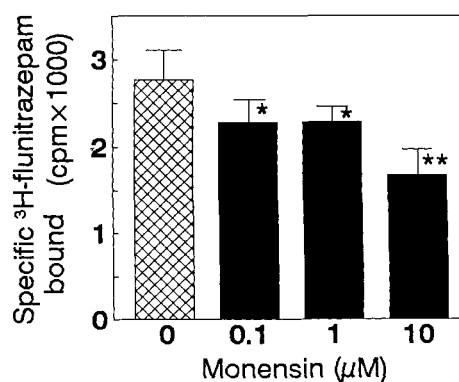


Fig. 1. Changes of the binding of <sup>3</sup>H-flunitrazepam to neuron culture in response to monensin treatment. Specific binding values were presented as cpm  $\pm$  SD. *n* = 15. \*: significantly different from control (monensin concentration 0) as calculated from student's *t* test, *P* < 0.01, \*\*: *P* < 0.001.

**TABLE I. Effect of Monensin on the Indicated Parameters of Neuronal Cultures**

| Effect of monensin on $^3\text{H}$ -FNZ binding <sup>a</sup>             |             |                 |                 |                |
|--|-------------|-----------------|-----------------|----------------|
| Monensin ( $\mu\text{M}$ )   | 0           | 0.1             | 1               | 10             |
| $^3\text{H}$ -FNZ binding  | 100%        | 82 $\pm$ 9.8%   | 82.4 $\pm$ 6.8% | 60 $\pm$ 14.4% |
| Effect of monensin on cellular distribution of the receptor <sup>b</sup> |             |                 |                 |                |
| $^3\text{H}$ -FNZ binding  | Con + Try   | Mon + Try       | Cyclo + Try     |                |
|  | 42 $\pm$ 7% | 40.3 $\pm$ 7.6% | 38.9 $\pm$ 5%   |                |

<sup>a</sup>Cells were treated with or without (control) monensin followed by binding assay.

<sup>b</sup>Cells were trypsinized (+Try) following exposure to monensin (Mon) (10  $\mu\text{M}$ ) or cycloheximide (Cyclo) (40  $\mu\text{g}/\text{ml}$ ). Con: control. Percentages of trypsin-resistant binding were determined.  $^3\text{H}$ -flunitrazepam (FNZ) binding assay and trypsinization of the cultures were performed as described in Materials and Methods. Values represent means  $\pm$  standard deviations (SD) normalized to control.

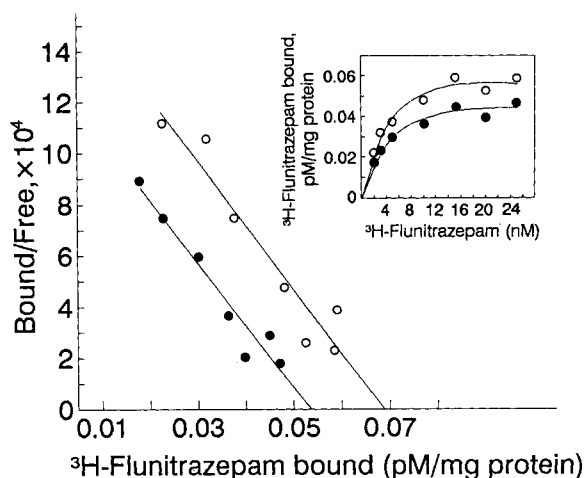
control level after removal of the drug. Saturation binding assay on the 1  $\mu\text{M}$  monensin-treated cultures showed that the number of  $^3\text{H}$ -FNZ binding sites was reduced ( $B_{\text{max}}$ : control = 0.069, monensin-treated = 0.053 pmole/mg protein) with no significant changes on the affinity of the receptor (Fig. 2).

Following trypsinization of control cultures, the binding of  $^3\text{H}$ -FNZ was reduced to 42  $\pm$  7% of control. Trypsinization of the cultures in the presence of monensin or cycloheximide showed similar percentages of trypsin-resistant binding sites; this indicated a consistent pool of trypsin-resistant receptors and the distribution of surface and intracellular receptors was not affected by monensin (Fig. 3, Table I). In comparison with monensin, treatment of the culture with cycloheximide did not change significantly the  $^3\text{H}$ -FNZ binding (Fig. 3).

To examine the effect of monensin on general cellular protein synthesis and glycosylation, the cultures were incubated with radiolabeled leucine or sugars and monensin. As shown in Figure 4, the incorporation of  $^3\text{H}$ -leucine into TCA-precipitable material was inhibited by about 15%, 26%, and 57% in the presence of 0.1, 1, and 10  $\mu\text{M}$  of the drug, respectively. By contrast, the  $^3\text{H}$ -mannose incorporation into TCA-precipitable proteins was elevated at all concentrations of monensin, with a significant increase of about 50% at 1  $\mu\text{M}$  monensin. Whereas the incorporation of  $^3\text{H}$ -galactose was reduced by approximately 22%, 59%, 56%, and 54% in the presence of 0.01, 0.1, 1, and 10  $\mu\text{M}$  monensin, respectively (Fig. 5).

## DISCUSSION

Treatment with 0.1 or 1  $\mu\text{M}$  monensin for 3 h reduced the number of  $^3\text{H}$ -FNZ binding sites on neuron cultures by about 18%. The kinetics of



**Fig. 2.** Monensin treatment induces a decrease in the number of  $^3\text{H}$ -flunitrazepam recognition sites. Reversible binding to the cells was performed following 3 h exposure to 1  $\mu\text{M}$  monensin. The specific binding data were analyzed and plotted according to the method of Scatchard. The inset shows the saturation binding of  $^3\text{H}$ -FNZ. The lines represent computer analyzed 1-site fit to the data.  $\circ$ : control,  $\bullet$ : monensin-treated. The  $K_D$  was 4.0 nM and 4.1 nM for control and monensin treated, respectively.

the degradation of  $\text{GABA}_A/\text{BZDR}$  has been proposed as a biphasic model, in which 42% of the receptor is degraded with a half life of 3.8 h, whereas 58% of the receptor is degraded with a half-time of 32 h (Borden et al., 1984; Czajkowski and Farb, 1989b). Therefore, approximately 19.3% of the total receptors undergo degradation following a 3 h period; this indicated that the replenishment of receptor protein was almost entirely hampered following the 3-h treatment with monensin.

$^3\text{H}$ -leucine incorporation by the culture was inhibited by about 15% and 26% in the presence of 0.1 and 1  $\mu\text{M}$  monensin, respectively. The studies regarding the secretion of thyroglobulin

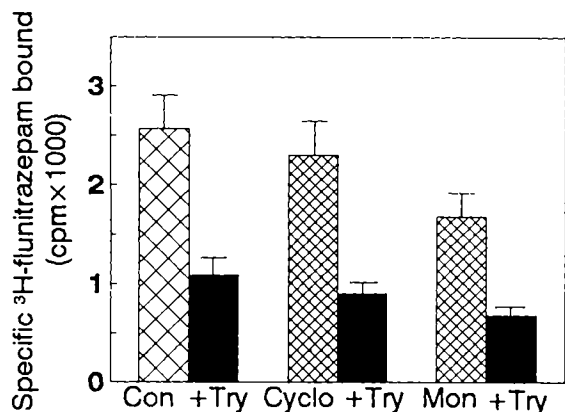


Fig. 3. Monensin and cycloheximide did not change significantly the distribution of cell surface and trypsin-resistant receptors. The cultures were preincubated with 10  $\mu$ M monensin (Mon) or cycloheximide (Cyclo) followed by trypsinization (+Try) and binding assay. Specific binding of <sup>3</sup>H-FNZ was shown as cpm  $\pm$  SD. con: control; n = 12.

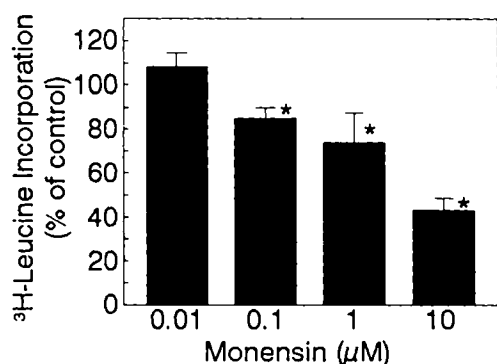


Fig. 4. Effect of monensin on incorporation of <sup>3</sup>H-leucine into acid-precipitable materials. The cultures were incubated with indicated concentrations of monensin and the radiolabeled amino acid. The incorporation of <sup>3</sup>H-leucine was determined according to the protocol in Materials and Methods. n = 4. \*: significantly different from control, 0.01 < P < 0.02 for 0.1  $\mu$ M, 0.02 < P < 0.05 for 1  $\mu$ M, 0.001 < P < 0.01 for 10  $\mu$ M from student's t test.

and events of retina have also exhibited decreases in the amino acid incorporation caused by monensin, although no alterations have been reported in other investigations (Fliesler and Basinger, 1987; Ring et al., 1987; Hamerschlag et al., 1982). It has been shown that reappearance of the GABA<sub>A</sub>/BZDR requires de novo protein synthesis after photoinactivation of the receptor in cultured neurons (Borden et al., 1984). Hence the decrease in general cellular protein synthesis reflects a partial reduction in the newly synthesized receptor proteins, which make up 4% of the total receptor as reported previously (Czajkowski and Farb, 1989b). One

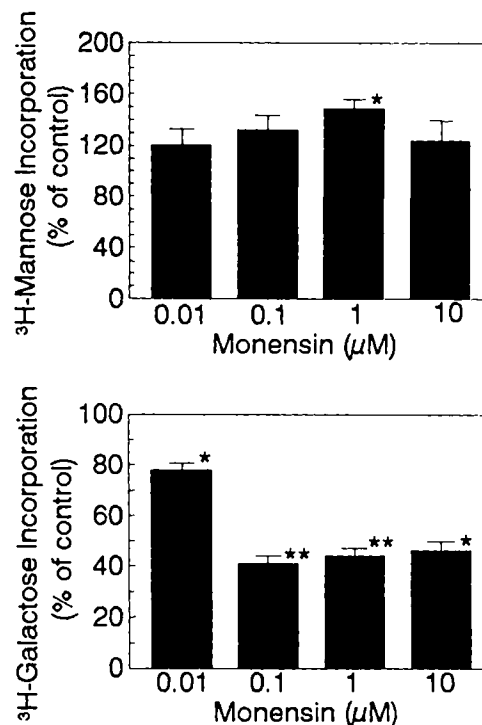


Fig. 5. Effect of monensin on incorporation of <sup>3</sup>H-mannose and galactose by the culture. The experimental procedures were described in Materials and Methods. n = 4. \*: significant difference from control, 0.01 < P < 0.02, in "Mannose Incorporation." \*: 0.001 < P < 0.01 and \*\*: p < 0.001, in "Galactose Incorporation."

other possible source for the blockade in <sup>3</sup>H-FNZ binding evoked by monensin came from a marked inhibition of galactose incorporation into glycoproteins.

Galactose has been shown to be one of the terminal sugars to be added to glycoproteins in the trans compartment of Golgi apparatus (Griffiths et al., 1982). Morphological effects of monensin on cultured neurons consisted of an initial swelling of the trans side of Golgi apparatus. However, the expansion of Golgi cisternae and vacuoles is time-dependent; by 3 h the cells are filled with large vacuoles (Yin and Yang, 1991). Monensin is also known to halt the translocation of newly synthesized proteins, such as viral membrane proteins, immunoglobulins, and fibronectin through the Golgi apparatus and to the cell membrane (Uchida et al., 1979; Strous and Lodish, 1980; Tartakoff et al., 1981; Griffiths et al., 1983). Thus one portion of the newly synthesized GABA<sub>A</sub>/BZDR proteins which had not acquired the binding capacity to <sup>3</sup>H-FNZ might be detained in a location proximal to the trans compartment of Golgi complex in the pres-

ence of monensin, causing a decrease in  $^3\text{H-FNZ}$  binding. However, additional possible mechanisms may account for the reduction of the  $^3\text{H-FNZ}$  binding caused by monensin, such as an increased rate of receptor degradation which was proposed to lead to the down-regulation of  $\text{GABA}_A/\text{BZDR}$  induced by chronic agonist exposure (Roca et al., 1990). It has been pointed out that receptor recycling was inhibited by monensin as revealed from studies on the mannose-glycoprotein receptor of macrophages (Wileman, 1984). Moreover, inactivation of the recycling receptors was suggested for producing a decrease in the number of galactosyl receptors in monensin-treated rat hepatocytes (Fiete et al., 1983; McAbee et al., 1991).

$^3\text{H-mannose}$  incorporation by the culture, in our study, was found to be increased by monensin, with a significant raise by about 50% at 1  $\mu\text{M}$ . Similar results have been obtained from studies on dopamine  $\beta$ -hydroxylase, fibronectin, and insulin receptor, in which the high-mannose form of these molecules was accumulated in the presence of monensin (Ledger et al., 1983; Jacobs, 1983; Kuhn et al., 1986). Thus, it appears that the addition of mannose to proteins occurs in a position proximal to the site of monensin action in cultured neurons.

When cultures were exposed to a higher concentration of monensin, i.e., 10  $\mu\text{M}$ , the  $^3\text{H-FNZ}$  binding was blocked by about 40%, with concomitant decreases, of 57% and 54%, in incorporation of radiolabeled leucine and galactose, respectively. Although the decreases in protein synthesis and galactosylation did not seem to block the entire population of newly synthesized receptor, the 40% reduction in binding was more than the 19.3% degradation in normal turnover of the receptor, suggesting an enhancement of receptor degradation. Whether or not this effect is reversible has not been observed. However, the cells may undergo overall degeneration because of the exposure to high concentration of the drug.

It was reported previously that approximately 42% of the  $^3\text{H-FNZ}$  binding remained after trypsinization of the cells, in which 20% was intracellular or sequestered and 20% was found to be associated with a 24 KD fragment generated by trypsin (Czajkowski and Farb, 1986). In the present study, a similar percentage of trypsin-resistant  $^3\text{H-FNZ}$  binding was observed, whether from control, monensin, or cycloheximide-treated cells; this indicated that the distri-

bution of surface and intracellular receptors was not altered by monensin. The observation that monensin induced galactose receptor inactivation but not redistribution in rat hepatocytes corresponds to our results (McAbee, 1991).

The data suggest that monensin impedes partially the synthesis and glycosylation of the  $\text{GABA}_A/\text{BZDR}$ , resulting in a reduction of the binding sites of  $^3\text{H-FNZ}$  to the receptor. Thus, it is likely that monensin obstructs the transport of  $\text{GABA}_A/\text{BZDR}$  from the Golgi apparatus to the intracellular pool and cell surface. It has been reported that the potentiation of  $^3\text{H-muscimol}$  binding by benzodiazepines disappears following removal of galactose from  $\text{GABA}_A/\text{BZDR}$  (Kuriyama and Taguchi, 1987). In addition, the glycosylation of nicotinic acetylcholine receptor is required for its maturation in attaining the binding ability to  $\alpha$ -bungarotoxin and the unglycosylated receptors are quickly degraded (Blount and Merlie, 1990). It is possible that galactosylation is related to the maturation and normal function of the  $\text{GABA}_A/\text{BZDR}$  as assayed by  $^3\text{H-FNZ}$  binding.

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#### REFERENCES

- Barnard EA, Darlison MG, Seeburg P (1987): Molecular biology of the  $\text{GABA}_A$  receptor: The receptor/channel superfamily. *Trends Neurosci* 10:502-509.
- Blount P, Merlie JP (1990): Mutational analysis of muscle nicotinic acetylcholine receptor subunit assembly. *J Cell Biol* 111:2613-2622.
- Borden LA, Czajkowski C, Chan CY, Farb DH (1984): Benzodiazepine receptor synthesis and degradation by neurons in culture. *Science* 226:857-859.
- Borden LA, Farb DH (1988): Mechanism of  $\gamma$ -aminobutyric acid/benzodiazepine receptor turnover in neuronal cells: evidence for nonlysosomal degradation. *Mol Pharmacol* 34:354-362.
- Costa E (1991): The allosteric modulation of  $\text{GABA}_A$  receptors. *Neuropsychopharmacology* 4:225-235.
- Czajkowski C, Farb DH (1986): Transmembrane topology and subcellular distribution of the benzodiazepine receptor. *J Neurosci* 6:2857-2863.
- Czajkowski C, Gibbs TT, Farb DH (1989a): Transmembrane topology of the  $\gamma$ -aminobutyric acid $_A$ /benzodiazepine recep-

- tor: subcellular distribution and allosteric coupling determined *in situ*. *Mol Pharmacol* 35:75–84.
- Czajkowski C, Farb DH (1989b): Identification of an intracellular pool of r-aminobutyric acid<sub>A</sub>/benzodiazepine receptors en route to the cell surface of brain neurons in culture. *Mol Pharmacol* 35:183–188.
- Fiete D, Brownell MD, Baenziger JU (1983): Evidence for transmembrane modulation of the ligand-binding site of the hepatocyte galactose/N-acetylgalactosamine-specific receptor. *J Biol Chem* 258:817–823.
- Fliesler SJ, Basinger SF (1987): Monensin stimulates glycerolipid incorporation into rod outer segment membranes. *J Biol Chem* 262:17516–17523.
- Griffiths G, Brands R, Burke B, Louvard D, Warren G (1982): Viral membrane proteins acquire galactose in trans Golgi cisternae during intracellular transport. *J Cell Biol* 95:781–792.
- Griffiths G, Quinn P, Warren G (1983): Dissection of the Golgi complex. 1. Monensin inhibits the transport of viral membrane proteins from medial to trans Golgi cisternae in baby hamster kidney cells infected with semliki forest virus. *J Cell Biol* 96:835–850.
- Hammerschlag R, Stone GC, Bolen FA, Lindsey JD, Ellisman MH (1982): Evidence that all newly synthesized proteins destined for fast axonal transport pass through the Golgi apparatus. *J Cell Biol* 93:568–575.
- Jacobs S, Kull FC, Cuatrecasas P (1983): Monensin blocks the maturation of receptors for insulin and somatomedin C: Identification of receptor precursors. *Proc Natl Acad Sci USA* 80:1228–1231.
- Jarvis DL, Oker-Blom C, Summers MD (1990): Role of glycosylation in the transport of recombinant glycoproteins through the secretory pathway of *Ledipteran* insect cells. *J Cell Biochem* 42:181–191.
- Kuhn LJ, Hadman M, Sabban EL (1986): Effect of monensin on synthesis, post-translational processing, and secretion of dopamine β-hydroxylase from PC12 pheochromocytoma cells. *J Biol Chem* 261:3816–3825.
- Kuriyama K, Taguchi J (1987): Glycoprotein as a constituent of purified r-aminobutyric acid/benzodiazepine receptor complex: structures and physiological roles of its carbohydrate chain. *J Neurochem* 48:1897–1903.
- Ledger PW, Nishimoto SK, Hayashi S, Tanzer ML (1983): Abnormal glycosylation of human fibronectin secreted in the presence of monensin. *J Biol Chem* 258:547–554.
- Maxfield FR (1982): Weak bases and ionophores rapidly and reversibly raise the pH of endocytic vesicles in cultured mouse fibroblasts. *J Cell Biol* 95:676–681.
- McAbee DD, Lear MC, Weigel PH (1991): Total cellular activity and distribution of a subpopulation of galactosyl receptors in isolated rat hepatocytes are differentially affected by microtubule drugs, monensin, low temperature, and chloroquine. *J Cell Biochem* 45:59–68.
- Mollenhauer HH, Morre DJ, Rowe LD (1991): Alteration of intracellular traffic by monensin: Mechanism, specificity and relationship to toxicity. *Biochimica et Biophysica Acta* 1031:225–246.
- Mohler H, Malherbe P, Draguhn A, Richards JG (1990): GABA<sub>A</sub>-receptors: Structural requirements and sites of gene expression in mammalian brain. *Neurochem Res* 15:199–207.
- Munson PJ, Rodbard D (1980): Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* 107:220–239.
- Olsen RW, Venter JC (eds) (1986): Benzodiazepine/GABA receptors and Chloride Channels: Structure and Functional Properties. New York: Alan R. Liss, Inc.
- Olsen RW, Tobin AJ (1990): Molecular biology of GABA<sub>A</sub> receptors. *FASEB J* 4:1469–1480.
- Ring P, Bjorkman U, Ekholm R (1987): Localization of the incorporation of <sup>3</sup>H-galactose and <sup>3</sup>H-sialic acid into thyroglobulin in relation to the block of intracellular transport induced by monensin. *Cell Tissue Res* 250:149–156.
- Roca DJ, Rozenberg I, Farrant M, Farb DH (1990): Chronic agonist exposure induces down-regulation and allosteric uncoupling of the r-aminobutyric acid/benzodiazepine receptor complex. *Mol Pharmacol* 37:37–43.
- Stephenson FA (1991): The GABA<sub>A</sub> receptors: Structure and function. *Curr Asp Neurosci* 3:177–193.
- Strous GJAM, Lodish HF (1980): Intracellular transport of secretory and membrane proteins in hepatoma cells infected by vesicular stomatitis virus. *Cell* 22:709–717.
- Sweetnam P, Nestler E, Gallombaro P, Brown S, Duman R, Bracha HS, Tallman J (1987): Comparison of the molecular structure of GABA/benzodiazepine receptor purified from rat and human cerebellum. *Mol Brain Res* 2:223–233.
- Tartakoff A, Hoessli D, Vassalli P (1981): Intracellular transport of lymphoid surface glycoproteins. *J Mol Biol* 150:525–535.
- Tartakoff A, Vassalli P (1979): Plasma cell immunoglobulin M molecules. *J Cell Biol* 83:284–299.
- Uchida N, Smilowitz H, Tanzer ML (1979): Monovalent ionophores inhibit secretion of procollagen and fibronectin from cultured human fibroblasts. *Proc Natl Acad Sci USA* 76:1868–1872.
- Vicini S (1991): Pharmacologic significance of the structural heterogeneity of the GABA<sub>A</sub> receptor-chloride ion channel complex. *Neuropsychopharmacology* 4:9–15.
- Wileman T, Boshans RL, Schlesinger P, Stahl P (1984): Monensin inhibits recycling of macrophage mannose-glycoprotein receptors and ligand delivery to lysosomes. *Biochem J* 220:665–675.
- Yin HS, Yang MF (1991): Effect of monensin on neuronal ultrastructure and endocytic pathway in cultured brain neuron. *Cell Mol Neurobiol*, In press.