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Overview

Are Astroglial Cells Involved in Morphine Tolerance?

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Morphine gives rise to a cascade of events in the nervous system affecting, among others, neurotransmitter metabolism. Tolerance develops for various effects shortly after administration of the drug. Also, physical dependence develops and can be demonstrated by precipitation of withdrawal reactions. Biochemical events in nervous tissue have been extensively studied during morphine treatment. This overview will focus upon brain protein metabolism since macromolecular events might be of importance for development of long-term effects, such as tolerance and physical dependence. Both dose- and time-dependent changes in brain protein synthesis and the syntheses of specific proteins have been demonstrated after morphine treatment, although methodological considerations are important. Different experimental models (animal and tissue culture models) are presented. It might be interesting to note that astroglial protein synthesis and the secretion of proteins to the extracellular medium are both changed after morphine treatment, these having been evaluated in astroglial enriched primary cultures and in brain tissue slices. The possibility is suggested that proteins released from astroglial cells participate in the communication with other cells, including via synaptic regions, and that such communication might be of significance in modifying the synaptic membranes during morphine intoxication.

KEY WORDS: Abstinence; astroglial primary culture; extracellular proteins; fluid diet; morphine; morphine intoxication models; naloxone; physical dependence; protein synthesis; tolerance.

GENERAL INTRODUCTION

Narcotic analgetics are interesting compounds, since repeated administration of these drugs produces a well-defined and easily quantified adaptive response, namely tolerance development. Physical dependence is thought to be coupled to tolerance (21, 24, 108), and physical dependence can be quantified by the precipitation of withdrawal symptoms (see *e.g.* 99). Understanding the mechanisms of these adaptive phenomena could serve as a model for understanding mechanisms of other adaptive behavior unique to central nervous system function.

A number of studies have been performed using biochemical and electrophysiological methods to elucidate the molecular mechanisms underlying these phenomena (for reviews, see *e.g.* 14, 15, 23, 24, 31, 66, 121). One drawback with this previous work is the many experimental models used with different methods for drug administration, different drug doses and different intoxication times. Varying experimental results have been reported and, in some cases, these are completely contradictory.

This overview will focus upon the benefits and drawbacks with experimental models such as tissue culturing and with different animal models. A literature survey of brain protein synthesis is presented, including the formation of specific protein fractions after morphine treatment. It seems that specific changes in protein metabolism take place after morphine treatment, although methodological considerations are important.

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Aspects of Morphine Action and Specificity

Morphine exerts a greater number of pharmacologic effects in vivo, and the production of analgesia is the most important from a medical point of view. Its widespread functions involve interactions with different neurotransmitter systems (see e.g. 11, 82) and, in addition, morphine interacts with the endogeneous opiates (73) and probably also with the other neuropeptide systems.

After ingestion, 10–15% of administered morphine is absorbed into the blood and a further similar amount is later absorbed from the caecum (63, 124). Morphine passes the blood-brain barrier and thereby can interact with astroglial cells. Until now, no detailed knowledge has been presented of the cellular events taking place. There are no data indicating an active uptake of morphine into astroglial cells (see 50, 100), so it might be assumed that the drug will distribute in the extracellular space. Only a minor fraction of the blood morphine level is found in the nervous tissue (see e.g. 35). Morphine interacts with μ-receptors and gives rise to a hyperpolarization of the neuron (91, 133). There are a number of immediate neurophysiological and biochemical events in the cell and its surroundings, much of which is at present unknown. In addition, subsequent activation of other neuronal circuits takes place with a cascade of both electrophysiological and biochemical events. Because of these effects, it is important to define the characteristics for specific opiate effects (Table I).

Adaptive responses already start developing some hours after a single morphine dose (71, 136) and increase in strength with repeated administrations. Tolerance can be regarded as a state of reduced responsiveness to a drug, arising from exposure to that drug. Physical dependence is a state of drug dependence where abstinence symptoms can be precipitated by drug withdrawal or by administration of antagonists (see 66). These adaptive changes, also including active drug seeking, limit the medical use of morphine as an analgesic and present the risk for abuse (aspects on the complexity of morphine effects and adaptation are summarized in Table II).

Table I. Characteristics for Specific Opiate Effects

—the effect(s) should be blocked by antagonist
—the effect(s) should be induced by agonists other than morphine (*e.g.* endogeneous opiates)
—the effect(s) should require stereospecificity (inactive isomers should not induce the effect(s))

Table II. Aspects on Mode of Narcotic Analgesic Drug Action

acute effect	primary "specific" (receptor-mediated) → secondary effects
	primary "non-specific" (non-receptor-mediated) → probably secondary unspecific effects
adaptation	"primary" tolerance (cellular/multicellular?)[1] (*i.e.* tolerance to pharmacological effects induced by stimulating the opiate receptor)
	"secondary" adaptation (adaptation of neuronal systems, not primarily involved in opiate, receptor-mediated pharmacological effects)
"mechanism of memory"	cellular[1]?
	multicellular[1]?

[1] cellular = coupled to specific neurons and surrounding astroglial cells

Aspects on Tolerance and Physical Dependence

Tolerance to narcotic analgesics is a phenomenon about which much has been written from a qualitative, descriptive, quantitative and philosophical point of view. Many hypotheses on the underlying mechanisms have been proposed (see Introduction). These include the development of so called "silent receptors" (22), the production of a peptide which antagonizes morphine action (20), the development of some form of immunological mechanism, the adaptation of the adenylate cyclase/cAMP system (109) or the interaction of opiates with enzymatic processes in the cells leading to a modification of neurohumoral factors (40, 114). Endogeneous opiates have also been implicated in the theories of tolerance (73). However, until now no theory has been able to explain all facets of tolerance or physical dependence, neither has it been possible to experimentally verify any theory in all its details.

Many theories of opiate tolerance require the

synthesis of proteins, new or already existing in the nervous tissue (see *e.g.* 13, 57, 78). The synthesis of proteins definitely seems necessary since the development of tolerance or physical dependence can be suppressed by inhibitors of protein or RNA synthesis without affecting the acute effects of opiates (see *e.g.* 6, 26, 27, 81, 102, 116). Attempts have been made to identify and isolate the protein or proteins affected by morphine treatment. So far, however, the results from many laboratories have been negative. In fact, there is disagreement on whether or not there is really an increase in protein synthesis after morphine treatment as many investigators have demonstrated a decrease (see Brain protein metabolism and the synthesis of specific proteins during morphine action). In the light of the structural and functional heterogeneity of cell elements even in small brain regions, macromolecular changes related to opiate treatment may simply average out in cell metabolism, leaving few remaining detectable changes.

Many attempts have been made to modify the opiate molecule and thereby separate the analgesic effect from that of tolerance. So far, experiments along this line have been unsuccessful. In fact, it seems as if the length of time that the receptor is occupied by the agonist is critical for the development of tolerance. It should be noted that even endogeneous opiates with modifications to give long-lasting effects produce tolerance (see *e.g.* 85, 123, 127, 130). Thus, the organism seems to become tolerant to, and probably dependent on, its own hormone-like neuropeptides. There also seems to be a cross-tolerance against the electrical stimulations that produce analgesia (83), suggesting that tolerance is a normal regulatory mechanism of some neural systems.

It has been discussed whether or not tolerance is a "cellular" phenomenon, thus restricted to events within or on a single cell and whether such tolerance requires many neuronal circuits. There are at least three experimental model systems which have provided evidence for tolerance being a cellular event, namely: neuroblastoma-glioma hybrid cells (70, 109, 110, 122), myenteric plexus of isolated guinea-pig ileum (43, 87, 131) and recording the discharge of nerve impulses from single cortical neurons of naive and morphine-dependent rats (104). However, it presently remains unsolved as to whether tolerance and physical dependence are dissociated phenomena at the molecular level (see *e.g.* 2, 24, 107). Several possible mechanisms could account for the parallel development of tolerance and dependence in situ.

If tolerance is a "cellular" phenomenon, and in spite of the effort being spent on elucidating neurochemical events underlying tolerance with all previous meager results, it might be that no single biochemical process is responsible for tolerance development but rather that several metabolic steps are involved. In consideration of the complex biochemistry of the "immunologic memory" and the participation of many cell elements, it might be hypothesized that not only neurons but also glial cells are involved in tolerance development.

Models to Study Biochemical Effects of Morphine on the Nervous System

Animal Models. The aim of our current drug research has been to define the changes and interactions resulting from the presence of drugs in the body in the hope of aiding medical science deal with the overwhelming social problems associated with drug usage and abuse. Although the problems associated with drugs are exclusively human, ethical considerations prevent investigators from conducting many important forms of research with human subjects. Therefore, experimental models are necessary if we are to understand how drugs act in the body. Such models must necessarily allow studies on the mechanism of action of drugs at the cellular level. While whole animal models permit several aspects of drug effects to be studied in the laboratory, certain experimental cell systems permit study of the cellular actions of drugs and these include work with whole tissues, cell types or subcellular fractions. These methods are useful in gaining knowledge about drugs, yet suffer drawbacks as models for studying the mechanism of action of drugs. Experiments performed in vivo can show effects of drug treatment on a particular system, correlating the biochemical with the behavioural parameters.

If we consider drug administration, a great number of models exist for morphine administration to laboratory animals, (particularly rats and mice). For the acute, single-dose administration, intraperitoneal, intravenous, intramuscular or subcutaneous routes are used mostly. For long-term intoxication, some of the most widely used methods are listed in Table III. This table also includes the benefits and general drawbacks with the various methods and references are given to authors who have made methodological contributions.

Table III. Animal Models for Long-term Morphine Intoxication

		References:
1) Injection (intraperitoneal, intramuscular, subcutaneous, intrathecal, intracerebral)		
Advantages: a) good absorption b) time for administr. can be determined—easy to regul. dose c) could be used as premedication in combination methods (see below)	Drawbacks: d) body weight decrease e) poor solubility of morphine—large volumes have to be used—tissue irritation and necrosis f) high top concentrations—uneven serum levels g) laborious	19, 25, 29, 34, 64, 77, 95, 126, 129, 134
2) Pellet implantation		
Advantages: a) perfect depot method when functioning	Drawbacks: b) repeated narcosis and surgery increase infection risk c) encapsulated pellets—lower serum levels—not suitable for intox. for more than 3–5 days	1, 6, 12, 61, 62, 72, 128
3) Oral intake A) Morphine in tap water		
Advantages: a) simple non-invasive method	Drawbacks: b) morphine has a bitter taste, even in low dose c) morphine solution has to replace water, and intake is directed by water need—does not function without premedication and/or force (for dependence) d) body weight loss	5, 9, 39, 69, 84
B) Morphine in sugar		
Advantage: The animal drinks to spontaneous dependence	Drawback: Calory-imbalance leads to body weight loss	30, 37, 94
C) Catheters, sonds with or without lever		
Advantages: a) Avoid bitter taste of morphine. b) even serum concentr. c) avoid calory imbalance	Drawbacks: d) Complicated equipment and performance, includ. narcosis e) learning of new behaviour is needed f) in some cases premedication, automatic, injection at onset of a "lever"—period g) gastritis, ulcus, stress	67, 115
D) Morphine in solid food (pellets) + morphine/sucrose solution.		
Advantage: a) Rapid dependence	Drawbacks: d) Complicated to measure food intake and thereby morphine intake c) laborious to avoid caloric imbalance	119, 135
4) Combination treatments Different types exist Body weight loss is a drawback		117

At our laboratory we have developed an ingestion model with morphine dissolved in a fluid diet (137). By pair feeding, intoxicated and control rats were ensured the same caloric intakes, nutrients and fluid. An interesting possibility with this specific model is that preference tests (the choice between a morphine diet and a control diet) can be performed on previously intoxicated animals under similar experimental conditions (Table IV).

Tissue Culture Models. Animal models often do not permit separation of cause-and-effect relationships. The reason for this is that treatment with drugs leads to altered metabolic and physiological states in the animal which are virtually impossible to control and can influence the results unknowingly. Subcellular studies, on the other hand, eliminate this interference by other systems yet often require conditions which do not exist in the intact cell. In addition, once the integrity of the cell has been destroyed, important functions and potential sites of action are lost from the study. Consequently, it is necessary to look for better model systems in order to understand cellular actions of drugs more clearly. Ideally, such systems should maintain the integrity and specificity of the whole tissue while providing the isolation and control of subcellular preparations. Cells grown in culture can provide some of these features. Despite the fact that cells in culture possess many attributes which would

Table IV. Advantages With Fluid Diet Intoxication Model (137)

—atraumatic, non-invasive
—caloric balance between intoxicated rats and controls (possibly pair-feeding)
—the animals rapidly become physically dependent
—easy to control individual total diet-, water- and morphine intakes
—no serum concentration tops over day and night
—no animals become ill or die
—the bitter taste of morphine is partially masked
—choice-tests with preference studies can be performed
  —choice-tests are performed under similar experimental conditions (similar cages, diet tubes etc) as intoxication
  —the animals receive the same calory- and water intake if they prefer morphine diet or control diet

Drawbacks with the intoxication model

—large morphine consumption
—in some experimental situations peroral morphine intake can be less suitable (should be combined with parenteral morphine administration)

make them an almost ideal system for the study of cellular actions of drugs, they have received little attention in this respect. Moreover, the types of cells which can be grown in culture are as many and varied as the organs from which they are originally derived. Established cell lines exist for many cell types. They are homogeneous and can be grown for long periods in culture. The drawbacks with these cells are that they are either transformed or contain oncogenic characteristics. Primary cultures, on the other hand, can be prepared from explanted or disrupted tissues. They originate from cells, tissues or organs taken directly from the organism and are regarded as primary until subcultivated. Primary cultures also provide the option of choosing tissue sources from the desired species, region and developmental age of the normal animal and the cells probably resemble more closely the cells found in vivo (see *e.g.* 49). They are good model system as an alternative to animal experiments, especially when studying the complexity of the brain. Primary cultures of the different brain cells, the neurons and their intimate glial neighbors, the astrocytes and the oligodendroglia, seem to be good model systems for studying toxicity and physiological effects of drugs. As primary cultures are generally heterogeneous concerning cell types, a detailed characterization of the cells expected to be present in the culture (see 44) and a functional characterization of their grade of differentiation are necessary (see 47). Furthermore, co-cultivation of *e.g.* neurons and astroglial cells (48) provides an interesting model for studying intercellular communication between these cells (see 52).

Brain Protein Metabolism and the Synthesis of Specific Proteins During Morphine Action

Protein synthesis is a central process for all cellular activity. Energy and various biochemicals of intermediate metabolism are utilized for the synthesis of proteins. The proteins are synthesized both for internal consumption by the cell and for export (*e.g.* hormones or serum proteins). In the nervous system, neurons and glial cells are those cells responsible for the main part of protein synthesis. The synthesis involves a complex series of reactions, from transcription of the DNA to RNA and a subsequent translation of the information to protein. The individual reactions are influenced by *e.g.* 1) protein concentration, pool size and distribution, 2) hormonal state, 3) nutritional state, 4) environment and 5) state of health of the animal. In the experimental model system, changes in protein synthesis can be influenced by other factors, that is, differences in amino acid uptake, compartmentation and reutilization. Furthermore, the route of precursor administration affects protein synthesis results from in vivo studies. For neural cells, the different routes of percursor administration are intravenous, intraperitoneal or intracerebroventricular. The drawbacks with the first two administration routes are that changes in blood flow can affect the distribution of the precursor. With the latter administration technique, there will be a tissue concentration gradient from the ventricles outwards.

The protein composition of cells can be regarded as a "finger print" of the specific cell indicating the biochemical classification, the differentiation degree and the functional status, including the reaction(s) of the cell to environmental factors. Changes in protein synthesis in relation to external stimuli might be a sensitive and easily studied parameter for obtaining quantitative data on cellular metabolism. Changes in the amount or turnover of specific proteins, on the other hand, could indicate changes in the functional parameters of the cells.

Morphine has been shown to interfere with most of the different steps involved in protein synthesis. The results are not uniform as different cell and tissue material, administration routes, doses and times for intoxication have been used. The most prominent of these results are listed in Table V.

Table V. Morphine and Nervous Tissue Protein Synthesis—Results from Earlier Literature

Animal		Intoxication method			Intoxication time					Protein synthesis			
Rat	Mouse	Peroral	Inject.	Pellet implant.	Single dose	Multiple doses (days)	Final dose mg/kg b.w.	Time between last dose and decapitation	Labeling with radioactive amino acid (min)	Increased	No change	Decreased	References
x			x			5	60	2–24 h	30			x	16
x			x			5	30	15–180 min				x	17
	x		x	x	30	3–5	100 (single d.) 75 (long-term)	30 min 3–5 d. (long-term)	30		x		41
	x		x				100	15 min, 16 h, 65 h	4 h		x (mitoch.)	x (synapt.)	75
x			x			12 days	50, 100 mg	1, 4, 12, 24 or 48 h			x (RNA)		10
	rabbit		x			32 h	10, 20, 40	16 h	$15\frac{1}{2}$ h		x		36
	x		x	x			75 (long term) 10 or 30 (single doses)	1, 3, 24, 72 h 30 min (single dose)	10		x (long-term) x (single dose)	x (singl. d.)	53
x			x				40	12 h	30	x (microsomes in cereb hypoth) x (single dose)		x (mitoch. long-term)	80
	x		x (acute)	x (chron)			75 or 10, 30, 100 mg	2 h	30 min 24 or 48 h			x (RNA)	54
x			x			42 or 84	25	60 or 120 min	20, 60 or 120	x (several brain reg.)	x (liver kidney)		76
	x		x (infusion 7 h)				35/ h	1.5, 3.5, 6.5	60	x (biphas. eff.)		x (single dose)	86
	x		x				100	30 min		morphine binds to polysomes and protects from disaggregation			118
x				x			75		15, 30, 60, 180 min or 24 h	x (Mc and Mw fract.)		x (long-term)	55
x			x		x		40	2, 24 h	15, 30, 45, 60		x	x	79
	x			x		72 h	75			x (phosphoryl.)			89
x				x			75 mg		3 or 24 h	x (high m.w. prot. bands–SPM-L)	x (tot. incorp.)	x (some prot. bands–SPM-H)	56
	x			x			75	72		x (cell free system)			28
x				x		3 weeks	75	72–75				x (phosphorylation)	32
x				x		75		72 h	2 h	x (band 6)		x (band 12)	18
x			x				75	72 h				x (phosphoryl.)	88
	x			x			75	72 h		x (protein kinase)			59
x			x		x		10, 30	30–120 min	6 min		x	x (polypept. elongat.)	92
x			x				15	1–20 min	30 sec	biphasic effects on phosphorylation			132
x				x		48, 96, 168 h	75		6	x (secr. prot.)			93
x		x				x	280, 340	1, 2, 3 h	60 min	x (biphas. eff.)	x		98

Table VI. Characteristics of Experimental Set When Evaluating Neurochemical Correlates to Morphine Treatment

—Well-defined animal material—sensitive for morphine (homogeneous material) and with experiment possibilities to "enlarge" the biochemical changes towards tolerance (over time)
observe: morphine treated rats eat less than controls—could give rise to a relative undernutrition
—Well-defined, small brain regions—small groups of neurons/glia (microdissection)
—Separation of neuron from glial biochemical reactions (from small brain regions) (cell cultivation)
—Evaluation of dynamic (dose- and time-dependent) changes in brain cells after morphine treatment
—Separation of receptor-mediated effects of morphine from effects due to *e.g.* transmitter activation in other cell systems
In addition: other non-receptor-mediated effects should be taken into account.

One can conclude that 1) soluble and membrane bound proteins, and also extracellular proteins including cerebrospinal fluid, are affected by morphine, 2) there are different results from various laboratories, and 3), protein synthesis inhibitors inhibit development of tolerance/physical dependence without affecting acute morphine effects.

It is obvious that there are requirements with the experimental system to obtain specificity (Table VI). In addition, the results might well predict that there are no single changes in protein synthesis and/or in the synthesis of specific proteins. The changes are most likely of a dynamic nature, probably involving the communication and integration between cells. This is attractive since drug adaptation phenomena are dynamic in nature.

At our laboratory we have tried to cast some light on these problems. Our experimental system consists of three sets:

- primary cultures — astroglial enriched / neuronal containing
- slices — morphine applied in vivo / morphine applied to the slice
- in vivo

In addition to in vivo studies on animals, the examination of cerebrospinal fluid (CSF) in humans might be an interesting approach.

CSF circulation from the cerebral ventricles through the subarachnoid space is unique in mammals (4). The development of the subarachnoid space parallels the development of the neocortex and intellectual functions. Studies on intact mouse brain (58) have shown that extracellular proteins represent an exclusive compartment which is linked to CSF. Therefore, CSF might be a valuable source for studying the metabolic events in brain tissues, especially those of long duration. Extracellular fluid (ECF)/CSF might be one link between experimental and clinical research on the nervous system. Consequently, it is important to develop methods allowing determintion of specific biochemical parameters in both fluids. Something that would be especially valuable is if results from animal models and/or tissue culture experiments create possibilities for identifying "markers" in CSF, *e.g.*, for the degree of physical dependence (see *e.g.* 65) or for people with high physiological susceptibility to drug abuse.

Methodological difficulties exist in identifying specific proteins as they occur in extremely small amounts. Sensitive detection systems are important and these include:

- immunoabsorption of serum-like proteins and, thereby, enrichment of nervous tissue proteins (see 101)
- incorporation of radioactively labelled amino acid precursors into protein and detection/semiquantification by sensitive electrophoretic techniques can reveal the synthesis of specific proteins (see 52; 97)
- immunological methods for quantification of defined (isolated) proteins.

In conclusion, we propose that

1) Morphine has differential effects on protein synthesis in different brain regions depending upon the length of the time of prior administration (Figure 1).
2) Long-term morphine treatment sensitizes the protein synthetic machinery of the brain resulting in an increased incorporation of [^{3}H]valine into protein following acute injection of morphine to physically dependent animals. The dose- and time-dependent effects are demonstrated in Figure 2.
3) In primary astroglial cultures from rat brain there is a dose- and time-dependent effect of morphine on protein synthesis and their secretion into the incubation medium (Figure 3).
4) It is important to separate the effects of opiates on nervous tissue proteins from that of the general metabolism in order to increase the detection level of the analytical methods.

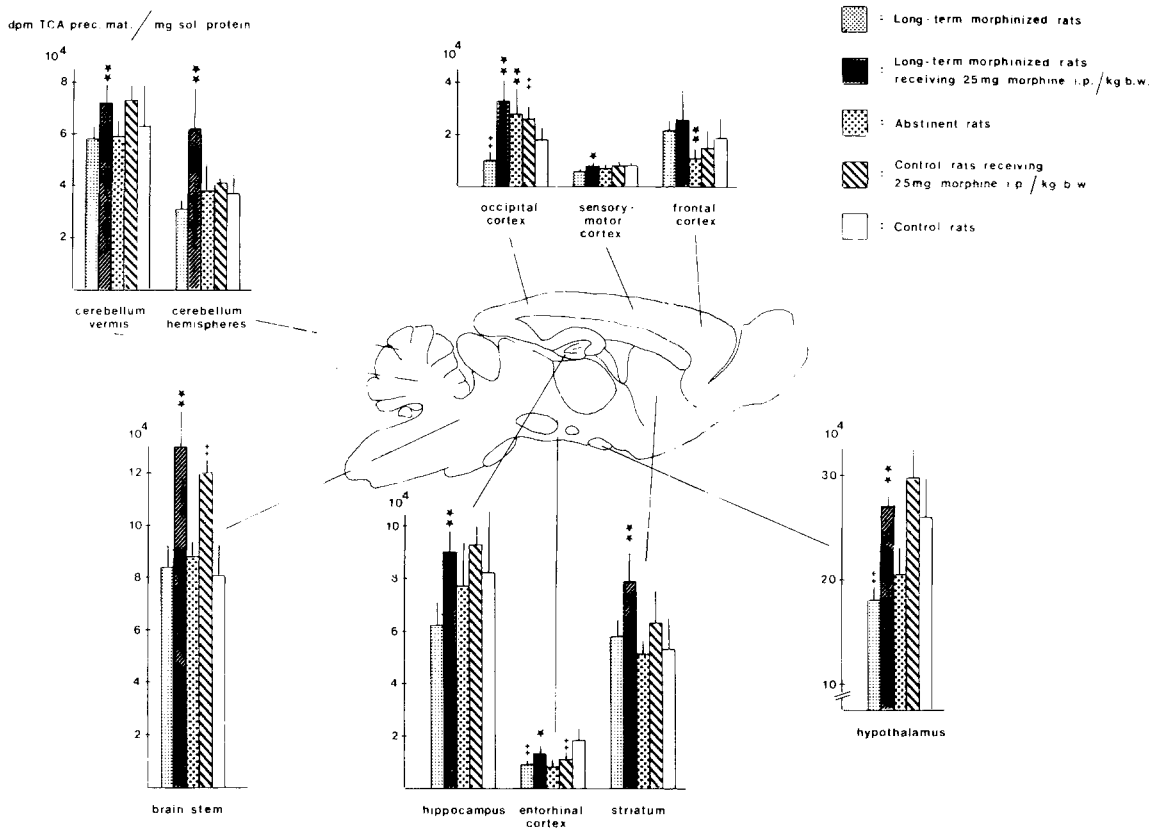


Fig. 1. In vivo incorporation of [^{3}H]valine into soluble protein from different rat brain regions. Five groups of rats were used: 1) long-term morphine intoxicated (13 days; final dose 340 mg/kg b.w./day p.o., according to 137 = ▦; 2) 25 mg morphine/kg b.w. injected i.p. into physically dependent (= ■) or 3) control rats (= ▨) two hours prior to decapitation, 4) morphine abstinent rats (99 – = ▩) and 5) controls (= □). Values are mean of six different animals ± SEM Student's *t* test was used.

* $p < 0.05$
** $p < 0.01$
† $p < 0.05$
†† $p < 0.01$

■ or ▩ vs. ▦

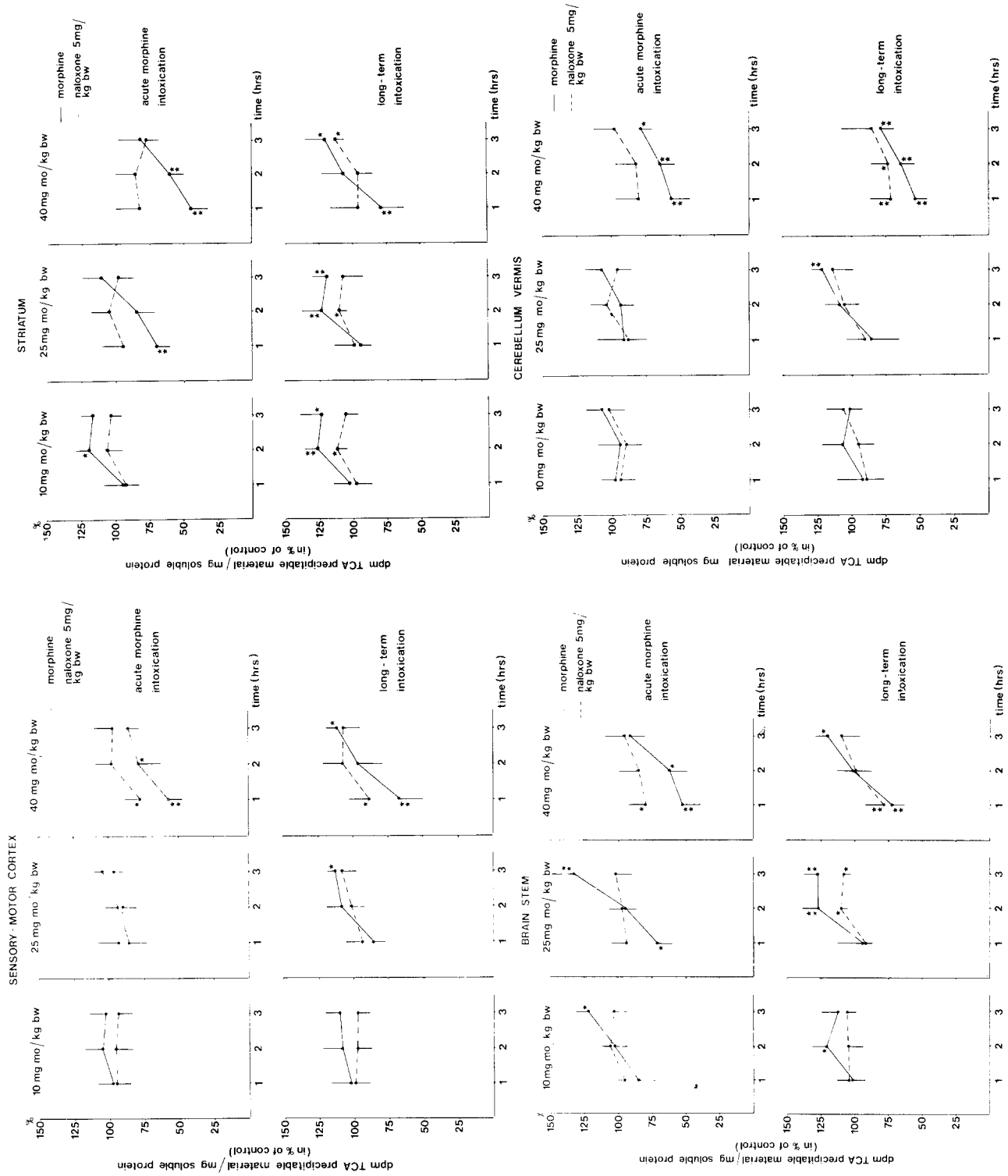
▩ or ▨ vs. □

Morphine exerts differential effects on protein synthesis in different brain regions (for details, see Rönnbäck and Hansson, 97).

Views and Speculations on Tolerance at the Cellular Level

At the cellular level, tolerance might be regarded as an adaptation to the drug. The physical dependence constitutes a modification of the metabolic machinery to restore cell functions to the pre-drug state while under influence of the drug. The phenomena include an identifying mechanism for the drug, *i.e.* memory. It might be that activation of a single "trigger pathway" related to the opiate receptor releases molecular modifications which give rise to an adapted state. However, it might also be that different metabolic pathways are "adapted" in parallel, thereby involving a great number of enzymatic systems. It might be hypothesized that the adaptive changes require the integration between different cells, *i.e.* astroglia and neurons. The reasons for this assumption are as follows:

Fig. 2a. In vivo incorporation of [^{3}H]valine into soluble protein from different rat brain regions. Protein synthesis was measured over time after a single morphine injection into control (= acute intoxication) or physically dependent (= long-term intoxicated – 13 days; final dose 340 mg/kg b.w./day p.o.) rats. (See 137). Morphine was administered i.p. in doses of 10, 25, or 40 mg/kg b.w. The precursor was injected into the cerebral ventricles immediately following administration of morphine, after 1 or 2 hr. Naloxone (5 mg/kg b.w. i.p.) was administered prior to morphine in some experiments. Incubation with the precursor amino acid was for 60 min. The results were expressed as indicated and each point represents the mean value of 4 experimental and 4 control animals ± SEM. Statistical analysis: Student's t test,
* $p < 0.05$; ** $p < 0.01$
Dose- and time-dependent effects on protein synthesis by morphine is demonstrated. (For details, See Rönnbäck and Hansson, 97).


STRIATUM
CEREBELLUM VERMIS
SENSORY - MOTOR CORTEX
BRAIN STEM
morphine
naloxone 5mg/ kg bw
10 mg mo/kg bw
25 mg mo/kg bw
40 mg mo/kg bw
acute morphine intoxication
long - term intoxication
time (hrs)
dpm TCA precipitable material / mg soluble protein (in % of control)

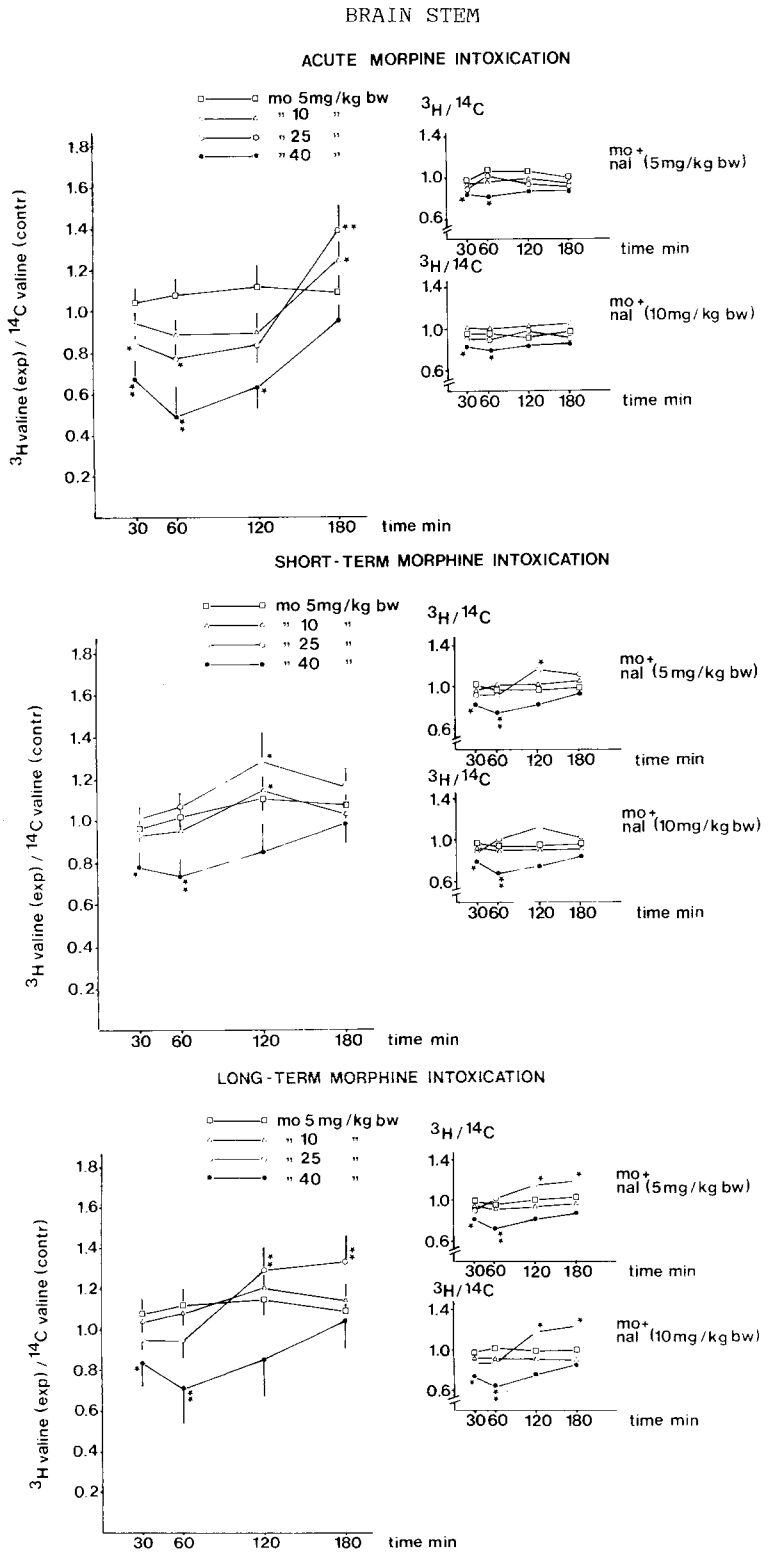


Fig. 2b. In vivo incorporation of $^3H/^{14}C$ (experimental/control) valine into soluble protein from brain stem of rat with different degrees of physical dependence on morphine. Control rats, short-term intoxicated (4 days, final dose 130 mg/kg b.w./day p.o.) or long-term intoxicated rats (13 days, final dose 340 mg/kg b.w./day p.o.) were used. The rats were injected i.p. with 5, 10, 25, or 40 mg/kg b.w. morphine and the labeled valine was administered as pulses for 30 or 60 min. The data are expressed as dpm ^3H/dpm ^{14}C ratio, normalized to 1.0 for control (^3H)/control (^{14}C) data by multiplying the dpm ^{14}C data by a factor of 3–5. Inset figures show results expressed in a similar way after 5 or 10 mg/kg b.w. naloxone, administered prior to the morphine. Each point represents the mean value of 3 different experimental animals and 3 controls (experiments in duplicate) ± SEM. (In the inset figures, SEM values are always less than 12%). Statistical analysis: Student's *t* test,
* $p < 0.05$; ** $p < 0.01$.
There seems to be a sensitization of the protein synthesis machinery in brain-stem with an increased incorporation of the amino acid precursor into protein following acute injection of morphine to physically dependent rats. (For details, see Rönnbäck and Hansson, 98).

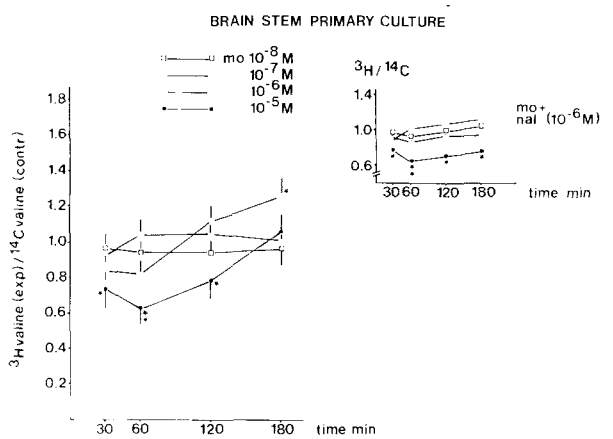


Fig. 3a. Incorporation of $^{3}H/^{14}C$ (experimental/control) valine into brain stem primary culture soluble protein after morphine administration in different doses. Naloxone was included in some experiments. Pulse-labeling with the valine was performed for periods of 30 or 60 min at various times after the drug exposure. The results are expressed as dpm ^{3}H/dpm ^{14}C ratio, normalized for control vs. control data to 1.0. Each point represents the mean value of 5 experimental and 5 control Petri dishes ± SEM. (When SEM are not shown, they are less than 12%). Statistical analysis: Student's *t* test,
* $p < 0.05$; ** $p < 0.01$.
Dose- and time-dependent effects on protein synthesis by morphine can be seen. (For details, see Rönnbäck and Hansson, 98).

1) All preparations where tolerance has been demonstrated as a "cellular" phenomenon include neuronal and glial material (43, 70, 87, 104, 109, 110, 122, 131).
2) Astroglial cells have a strategic position with cellular processes extending both towards the blood vessels and towards the synaptic regions. One astrocyte can have processes towards many synapses on one neuron, but also have processes towards synapses in different neuronal systems.
3) Astroglial cells have many functions necessary for the developing and mature nervous tissue, including involvement in synaptic events. A basis does exist for communication between astrocytes and other cells, *i.e.*, neurons. Astrocytes can be sensitive for presynaptic events as they have sites for active uptake of amino acid neurotransmitters (46, 105, 106) and at least diffusion uptake mechanisms for monoamines (46). They also have receptors for various neurotransmitters and neuromodulators (125). It is interesting to note that interactions exist between many neuropeptide and monoamine receptors, providing a basis for the cellular input of dynamic information.

The cells are K^+ and Cl^- sensitive (74) and, thereby, are sensitive even for postsynaptic events. Thus, they can participate in the regulation and restoration of synaptic events and having enzymes for neurotransmitter catabolism (42, 45). In addition, astrocytes have been shown to depolarize directly upon stimulation by neurotransmitter amino acids (7, 68) and probably also by adrenergic agonists (60). The cells also synthesize proteins, some of which are secreted (3, 50). It might be speculated that such proteins must be released when the cells are long-term stimulated and that some of them may facilitate communication with other cells, including in the synaptic regions (see *e.g.* 3, 98, 111). It might also be that astroglia modulate activity in different neuronal systems.

4) Glial metabolism, with changes in protein synthesis and changes in biochemistry of single nerve tissue-enriched proteins, have been implicated in the acquisition of new behaviors (see 112–113).

Varying results exist concerning the basis of morphine influence on astroglial cells. Rougon et al. (103) suggested the presence of opiate receptors inhibiting the noradrenaline-mediated, activation of adenyl cyclase in astrocytes of cerebral cortex, while van Calker and Hamprecht (125) and Hansson and Rönnbäck (50) were unable to demonstrate any receptors in astrocyte membranes coupled to cAMP production. Nor did Hansson and Rönnbäck (50) find any active [^{3}H]morphine uptake, while they did find changes in astroglial protein synthesis after exposure to morphine in physiological concentrations, suggesting the presence of some form of recognition sites for morphine. Such results are supported by the work of Bunn et al. (8) who provided evidence for a kappa opioid receptor on pituitary astrocytes, and Pearch et al. (90) who suggested an interaction between noradrenaline and opiates. In situ, it might also be plausible that the astrocytes receive information about the ionic changes and neurotransmitter effects caused by morphine.

When the hyperpolarization caused by morphine persists for several hours, it might be assumed that the astroglial cells respond in order to modulate and restore synaptic events. It might be that the cells communicate with the neuron at the synaptic gap by secreting proteins which affect the pre- and postsynaptic membranes and which also affect cells at some distance to modulate and co-ordinate cellular circuits. Such macromolecules might directly change the neuronal membrane and/or induce

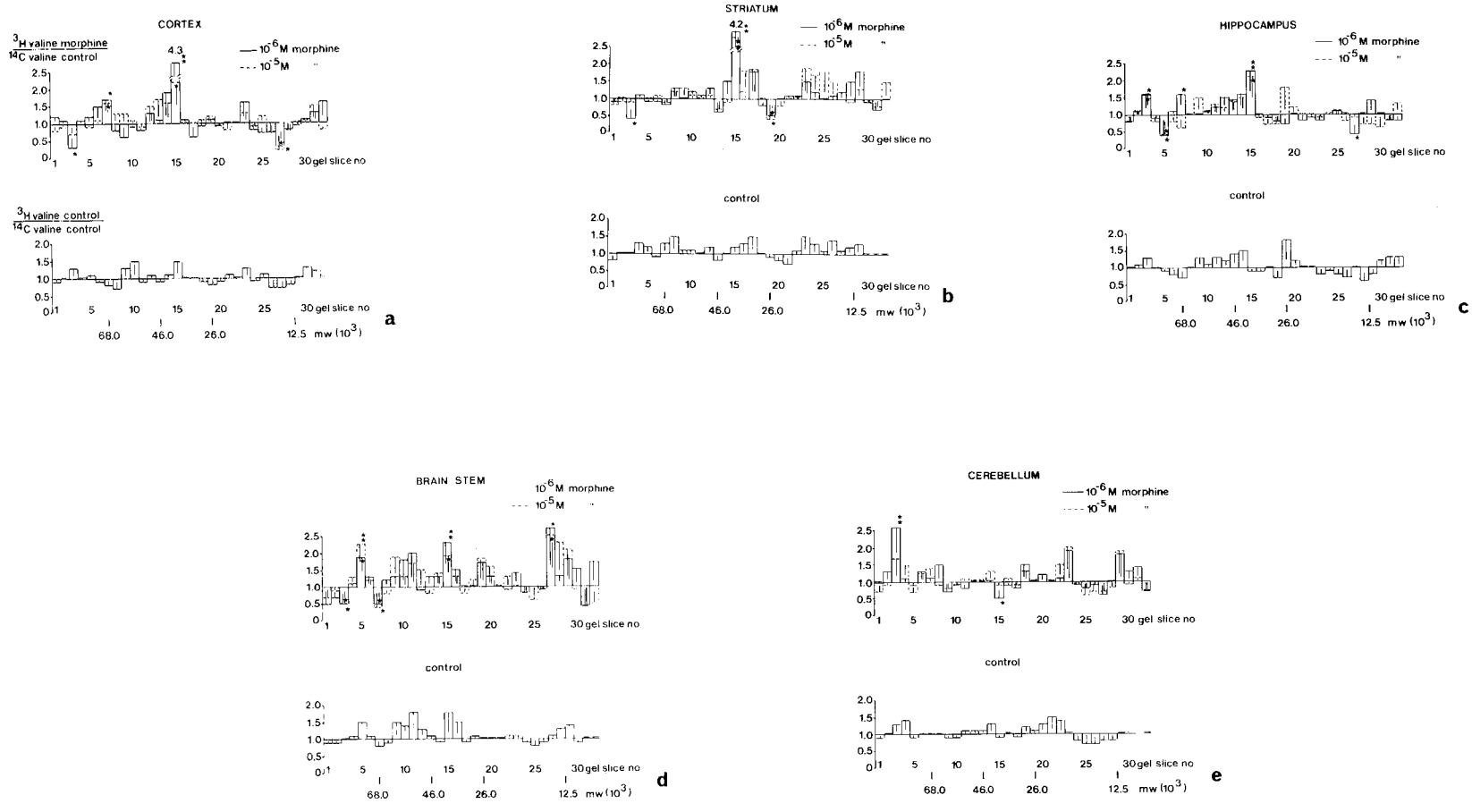


Fig. 3b. Electrophoretic separation (15% polyacrylamide gels in SDS) of $^{3}H/^{14}C$-valine labeled protein bands obtained from the incubation media of astroglial-enriched primary cultures from different regions of rat brain after morphine administration (10^{-6} or 10^{-5} M for 4 hr). The gels were sectioned into 32 slices and mw:s are indicated in the figure. Control experiments ($^{3}H/^{14}C$) were performed. Each point represents the mean of 4 different gels (material from 5 Petri dishes pooled in each). Statistical analysis: Student's *t* test comparing $^{3}H/^{14}C$ values of control/control slices.
*$p < 0.05$; ** $p < 0.01$.
There is a differential relative increase and decrease in the release of proteins/polypeptides into the incubation media from the various cultures after exposure to morphine. (For details, see Hansson and Rönnbäck, 51).

changes in the affected neuron towards a diminished effect of the drug, *i.e.* tolerance. When the drug is presented a second time, the biochemical machinery, including coding for protein synthesis, is adapted for a more rapid production of the proteins involved, thereby providing at least one cytomolecular mechanism for the "memory component" of tolerance/physical dependence.

When tolerance/physical dependence is looked upon in this way, it is obvious that some of the macromolecular changes that occur probably are the basis of cellular adaptation. Despite this, the single protein fraction(s) involved might appear in very low concentration(s). Conformational changes are always important to consider. Even dynamic events (over time) must be taken into consideration. If these speculations are true and tolerance requires interaction between astrocytes and neurons, studies of molecular mechanisms for tolerance and physical dependence to develop can be one way of enlightening earlier proposals of interactions between neurons and glial cells as the basis of "higher" adaptive functions of the brain (see 38, 96, 120).

Concluding Remarks

Morphine has its proven role in the relief of chronic pain. This effect is produced at different sites and levels in the nervous system, *e.g.*, spinal cord and cerebrum. Analgesia and other short-term effects are thought to be mediated by stimulating opiate receptors. Interactions of morphine and/or its metabolites with endogeneous opiates, and also with neurotransmitters and neuromodulators, do occur. Tolerance and physical dependence appear as long-term effects, *i.e.*, for some hours after morphine administration. When studying the biochemical correlates to such tolerance/dependence effects, it is important to choose the correct experimental model or combination of models. Tissue culture could be used to evaluate direct cell responses (neuron or glia from various brain regions) to morphine, while animal models also reveal the complex integrated cellular responses from the intact tissue, giving correlation possibilities between biochemical data and behaviour parameters. The effects of different morphine concentrations should be evaluated, but the effects should also be followed over time after morphine exposure.

Protein synthesis is necessary for cellular function and, therefore, is probably important for adaptive changes of nerve tissue. Intracellular protein composition is extremely complex, with numerous single protein entities existing in extremely low amounts with great functional significance. One approach for studying protein metabolism during morphine action is to evaluate the significance of the soluble proteins secreted into the extracellular medium. Astrocytes possibly contribute to such proteins which could participate in communication with other cells, including in synaptic regions.

If there are changes in specific protein fractions, acting as enzymes or in the communication with other cells during morphine action, which are associated with development of tolerance and/or physical dependence, the creation of inhibitors against such proteins would be one way to control the adaptive responses of opiates. In this way, opiates could be used more safely in the clinical treatment of chronic pain. In addition, if there are protein changes present in the CSF (or blood) of subjects (animals or humans) who rapidly develop an active drug craving after morphine administration, such proteins could be used as "markers" for these "susceptible" persons. Results along this line, of course, could have special significance to diminish abuse.

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