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Microdialysis in pain research

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Abstract

In vivo microdialysis has been used in preclinical pain research for more than a decade. This valuable tool allows correlations between nociceptive behavior and neurotransmitter release in pain-related CNS sites. However, several methodological issues must be considered to adequately interpret microdialysis data. Thus, the aim of this review is to describe key considerations, potential pitfalls, and important control experiments. We focus on animal experiments which evaluate the effects of noxious stimulation on CNS neurotransmitter release, particularly those that address clinically relevant problems in patients with long-lasting painful conditions.

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1. Introduction

Microdialysis refers to the filtration of water-soluble substances in the extracellular fluid through a dialysis membrane into a perfusion fluid, which is collected and analyzed for the substances of interest. This technique has been used extensively in preclinical neuroscience for almost 30 years (for review see [1–5]). Microdialysis allows on-line estimates of neurotransmitters in the living animal. Alternative *in vivo* methods for the study of neurotransmitter release are the ‘push–pull’ technique, which has been used in the brain [6], spinal cord [7] or intrathecal space [8]. Monitoring of receptor internalization [9] as an indicator of neurotransmitter release only permits one estimation at one time point per animal. With microdialysis, the possibility to correlate behavior with changes in the extracellular level of neurotransmitters in CNS regions involved in pain transmission and pain modulation is a great advantage. Increased knowledge of the neurotransmitters released in different pain models may result in the identification of important pharmacological targets. In this respect, animal models, which mimic the clinical situation, are of special interest. Hitherto, most microdialysis experiments in animal models of pain have investigated the effect of formalin or carrageenan injection into the hindpaw. However, the relevance of these models for chronic pain states in man may be questioned.

In this review, we present the basic requirements and technical aspects of microdialysis experiments in general and summarize the results obtained in animal

models of pain. The effects of pharmacological agents in the absence of noxious stimulus or of electrical stimulation on the release of neurotransmitters in control animals are only addressed to a minor extent.

2. Characterization of neurotransmitter release using microdialysis

As a model of *in vivo* neurotransmitter release, microdialysis rests on the assumption that the extracellular concentration accurately reflects the synaptic concentration. The problem with this assumption is that the extracellular concentration of a neurotransmitter is not only affected by neuronal release, but also by enzymatic metabolism, diffusion and reuptake. Furthermore, neurotransmitter substances found in microdialysate may have glial origin, indicated by calcium independent release [10]. Thus, changes in the microdialysate concentration of a neurotransmitter do not necessarily reflect synaptic neuronal release. Rather, they more likely reflect overflow from the synaptic cleft (for reviews on microdialysis techniques see [1,2]).

Control experiments can be performed to provide evidence that at least a certain portion of pain- or drug-induced changes in microdialysate levels of neurotransmitters result from changes in synaptic release. Such experiments might address the effects of sodium channel block and calcium depletion on the microdialysate concentration.

2.1. Potassium induced release

A common strategy to induce neurotransmitter release involves the inclusion of high concentrations of potassium ions in the microdialysis perfusion fluid. Passive diffusion of these ions across the dialysis membrane and into the tissues generates depolarization, leading to neurotransmitter release. In the dorsal horn, potassium stimulation has been demonstrated to increase monoamine, neuropeptide, and amino acid concentrations [11–18]. It must be kept in mind, however, that potassium depolarization can lead to release of amino acids not only from neurons, but also from glia.

In order to obtain a reproducible stimulation via the microdialysis probe, a concentration of 100 mM potassium in the perfusion medium is often required. This concentration may seem high to those researchers familiar with *in vitro* release studies. However, the concentration of potassium in the extracellular fluid surrounding the dialysis probe is probably closer to 10 mM [19], a concentration often used to stimulate release in release studies *in vitro*.

For example, in the halothane anesthetized rat, 100 mM potassium induced a significant four-fold increase of cholecystinin-like immunoreactivity (CCK-LI) in the dorsal horn, while 50 mM potassium induced only a slight non-significant elevation of the CCK-LI level and 25 mM potassium did not affect the level of CCK-LI in the microdialysate at all [11]. In the periaqueductal gray (PAG) in awake rats, potassium (100 mM) induced a more than 30-fold increase of the level of GABA [20] a 14-fold increase of neurotensin like immunoreactivity (NT-LI) [21] a two-fold increase of substance P-like immunoreactivity (SP-LI) [22] and no significant increase of glutamate [22]. Thus, there is great variability in the response to potassium stimulation. However, sometimes the basal neuropeptide concentration is below the detection limit of the assay. Under these circumstances the relative magnitude of potassium induced release may be underestimated.

2.2. Sodium channel dependent release

As an alternative to potassium stimulation a more specific activation of sodium channels by application of veratridine [23] (a neurotoxin, which opens sodium channels) may be used [16,24–28]. Conversely,

provided the release detected by microdialysis is secondary to sodium channel activation, administration of tetrodotoxin (TTX) [29], a sodium channel blocking agent should decrease the neurotransmitter level in the microdialysate or block stimulation induced release [10,13,16,27,28,30–35].

2.3. Calcium dependent release

With the exception of certain amino acids, which can be released by voltage-dependent but calcium-independent mechanisms [36], classical vesicular release of neurotransmitters is calcium dependent. Thus, perfusion of the microdialysis probe with low calcium and high magnesium concentrations should decrease neurotransmitter levels in the microdialysate (magnesium blocks calcium channels upon competition with the transport of calcium and competes with the calcium binding [37]). Instead of removing calcium from the medium, classical vesicular release mechanisms can be demonstrated by inclusion of calcium channel-blocking agents, such as verapamil or omega-conotoxin [30,38]. Both the spontaneous and the drug or potassium evoked release of neurotransmitters may be decreased in the absence of calcium [10,24,30,39].

However, calcium dependent spontaneous (basal) release [10,24] may be difficult to demonstrate due to technical limitations (e.g., basal levels close to the detection limit) [30], non-vesicular release [36], or a large contribution of glial derived neurotransmitter.

3. Further considerations for the design of microdialysis experiments

In addition to providing evidence that the microdialysate concentration of a transmitter substance is associated with neuronal release, other control experiments may need to be incorporated into a microdialysis project design. These include: use of *in vitro* recovery methods to quantify the percentage of neurotransmitter that crosses the dialysis membrane and the demonstration of a stable basal release (variability in baseline precludes many microdialysis experiments from reaching fruition). To obtain a stable basal release special measures to enhance detection of neurotransmitters often have to be taken. When, despite this, spontaneous baseline concen-

trations of neurotransmitter cannot be detected, strategies must be developed to evoke a continuous stable release or pulses of stimulated release. These evoked changes can then become the target for modulation by noxious stimuli or analgesic drugs.

3.1. *In vitro* recovery

For determination of *in vitro* recovery, microdialysis is performed in vials containing different concentrations of the transmitter under investigation. The *in vitro* recovery varies widely between transmitter substances and is also dependent on the fiber material. Due to the high molecular weight, the relative recovery (i.e., (concentration in the microdialysate/concentration in the outer medium) × 100%) of neuropeptides is generally only a few percent in contrast to the 10 or 20 percent recovery typically reported for amino acids and monoamines [40,41].

A stable linear correlation between the concentration of various neuropeptides in the microdialysate and the outer medium is a prerequisite for quantitative evaluation of the microdialysis data. However, it should be kept in mind that *in vitro* recovery only represents an approximation of the recovery of the microdialysis probe in the brain or spinal cord *in vivo*. *In vivo*, factors such as density of CNS tissue and blood flow in the surrounding tissues could dramatically alter the diffusion properties of any chemical substance [2]. This issue has been addressed with direct comparison of extracellular concentration with microdialysate concentration [19].

3.2. *Strategies to enhance detection of neurotransmitters in microdialysis*

3.2.1. *Monoamines*

Extracellular concentrations of 5-HT are primarily controlled by two mechanisms: reuptake into the presynaptic terminal and deamination by monoamine oxidase [42]. If monoamines or indoles cannot be detected in microdialysate samples, the addition of reuptake blockers or monoamine oxidase inhibitors to the perfusion fluid may result in measurable levels. However, inclusion of a reuptake blocker can alter the pharmacological mechanisms of neurotrans-

mitter release [35] and complicate the interpretation of the obtained results.

3.2.2. *Neuropeptides*

In contrast to monoamines and amino acid transmitters, with extracellular levels in the nanomolar range, the extracellular concentration of neuropeptides is in the picomolar range. For the detection of 'basal levels' (i.e., levels without stimulation) of neuropeptides in microdialysates, the addition of peptidase inhibitors to the perfusion medium is often needed. However, even with peptidase inhibitors, 'basal levels' may not be detected.

The rather poor temporal resolution of the microdialysis technique (e.g., collection of 10–30 min samples) may prevent the detection of short lasting pulses of neurotransmitter release. However, the apparently slow clearance of neuropeptides after neuronal release and the possibility that they may exert their effect far from the site of release via volume transmission [43] possibly make microdialysis especially appropriate for this class of messenger substances.

3.3. *Basal release*

The flow rate of the perfusion fluid can affect the concentration of substances in the microdialysate. Low flow-rates result in higher concentrations in the microdialysate [3]. This is of particular importance if the technique used for analysis, for example HPLC or CE-LIF, is optimized for a small sample size (10–20 μ l). For radioimmunoassay (RIA) larger sample volumes are normally used and the total amount of substance per sample may be more important than a high concentration in a minimum of volume. Thus, the flow-rate and the sampling interval can preferably be adjusted to yield the highest amount of peptide per sample. In studies on neuropeptide release we typically use a flow rate of 3.5 μ l/min and a sample size of 100 μ l.

Correct choice of timing of the surgical procedure and the microdialysis experiment may minimize variability. Immediately after insertion of the probe, the neurotransmitter levels are generally higher and level off during the first hours. Thus, a reasonable time should be allowed prior to the start of the microdialysis experiment. Some researchers prefer to

implant the probe or a guide cannula (see below under ‘brain microdialysis’) into the brain one or a few days prior to the microdialysis experiment. In addition, since stress may also affect the neurotransmitter release, the animals should have time to adapt to the experimental environment when studies are performed in awake animals [5,44]. In experiments on anesthetized animals the anesthetic agent may decrease or increase the neuronal release monitored by microdialysis [45–47].

Detection of a stable basal release (i.e., stable neurotransmitter levels the dialysate samples collected without stimulation) is not always possible. Before resigning oneself to the obvious possibility that there is no basal release to be detected, various technical problems should be considered, including: (1) unreliable pumps or leaking syringes; (2) a microdialysis membrane not optimal for the neurotransmitter under investigation [40]; (3) detection methods with insufficient sensitivity.

4. Methods of detection of neurotransmitters in microdialysate samples

4.1. HPLC (High-Performance Liquid Chromatography)

For the detection of amino acids and monoamines, HPLC coupled to an electrochemical or fluorometric detector is the most commonly used method [48–50]. In studies of nociceptive mechanisms glutamate and aspartate and PGE₂ have been detected in microdialysate obtained from the intrathecal space [51] and dorsal horn [17], GABA in the PAG [26] and in the dorsal horn [12]; 5-HT and noradrenaline in the dorsal horn [52,53] and noradrenaline in the locus coeruleus [54] and 5-HT in the RVM [10] (for details see Tables 1–4).

4.2. Capillary electrophoresis

A more sensitive technique for detection of amino acids in microdialysate is capillary electrophoresis with laser induced fluorescence detection (CE-LIF) [50]. This technique handles smaller volumes and for example a detection limit for GABA is 0.3 nM [20] as compared to 1 nM in a sensitive HPLC method

[55]). The high sensitivity enabled us to detect a decrease of the baseline level of GABA during local administration of morphine in the PAG [56]. A similar technique, capillary zone electrophoresis, has successfully been used to monitor the release of amino acids in the PAG in response to formalin injection [39].

4.3. Radioimmunoassay

For detection of neuropeptides in microdialysate, radioimmunoassays (RIA) have been the method of choice due to the potentially high sensitivity of this technique. By using small volumes, low radioligand and antibody concentration and sequential incubation technique, the sensitivity may be improved considerably compared to conventional RIA (for details see [57,58]). However, despite high sensitivity, detection of basal levels is not always possible. The following neuropeptides have been demonstrated by RIA in microdialysate obtained in the brain or spinal cord in studies of pain mechanisms: Substance P (SP) [52], cholecystokinin (CCK) [11], neurotensin [21,59] galanine [60], met-enkephalin [59] and beta-endorphin [61].

In addition to neuropeptides the concentration of prostanoids in microdialysate has been quantified by RIA [51,62]. Enzyme immunoassays have also been used successfully to detect prostanoids in microdialysate [63].

4.4. Mass spectrometry

For the detection of met-enkephalin and neurotensin in microdialysate samples mass spectrometry has been used as well [64].

Cannabinoids in microdialysates obtained from the PAG have been quantified by atmospheric pressure–chemical ionization mass spectrometry in [65].

5. Drug administration via the microdialysis probe

The microdialysis technique is not only suitable for monitoring the extracellular level of neurotransmitter candidates in the CNS, but also for local administration of pharmacological agents [66]. Dif-

Table 1
Acute pain models

Pain model	CNS region, species, awake/anaesthesia	Transmitter	Ref.
Plantar incision	Dorsal horn, rat, awake	Asp ↑, Glu ↑, Ser ↑, Asn ↑, Gln ↔, Gly ↔	[34]
Pinching or saline injection in the hind paw	PAG, rat, awake Nucleus accumbens, rat, awake	Glu ↑, Arg ↑, Asp ↑	[39]
		Dopamine ↑	[44]
Spinal capsaicin, spinal PGE2	Intrathecal, rat, awake	Glu ↑, Asp ↑, Tau ↑, Gly ↑, GABA ↑, Ethanolamine ↑	[97]
Intrathecal strychnine + hair deflection	Intrathecal Rat urethane–anaesthesia	PGE2 ↑	[98]
Phorbol ester into the dorsal horn	Intrathecal, rat, awake	Asp ↑, Glu ↑, Gly ↑, Tau ↑, Gln ↓, Tau ↓, Asn ↔, Ser ↔, Thr ↔, Ala ↔	[99]
Mustard oil injection into the temporomandibular joint	Spinal trigeminal nucleus, rat, chloralose or barbiturate anaesthesia	Glu ↑, Asp ↑, Ser ↑, Gly ↑	[100,101]
Electrical stimulation of A-delta fibers	Dorsal horn, rat Urethane-chloralose anaesthesia	5-HIAA ↑, DOPAC ↑, HVA ↑	[14]
Electrical high intensity stimulation of afferent A-delta or C fibers	Dorsal horn, rat Urethane-chloralose anaesthesia	Noradrenalin ↑	[15]
Electrical tetanic stimulation of the sciatic nerve (LTP induction)	Dorsal horn, rat, Halothane or urethane anaesthesia	SP-LI ↑	[102]

Glu: Glutamate; Asp: Aspartate; Gln: Glutamine; Asn: Asparagine; Ser: Serine; Cit: Citrulline; Arg: Arginine; Thr: Threonine; PGE2: Prostaglandin E2; 5-HIAA=5-hydroxyindoleacetic acid; DOPAC: dihydroxy-phenylacetic acid; HVA: homovanillic acid; ↑=increased release, ↓=decreased release, ↔ no change or blocked release.

ferent doses of a drug or a combination of agonists and antagonists can be administered in the same experiment without adding any fluid to the extracellular space. Indeed, many agents of interest for pain research including N-type calcium channel blockers [67], opioid receptor agonists and antagonists [30,68,69] and neurokinin-1 receptor antagonist [70] and nitric oxide synthase inhibitors [71] have been delivered directly into the dorsal horn. The dose of a drug administered by reverse microdialysis can be determined by comparing the total amount of a drug in the perfusion medium (prior to administration) minus the total amount of a drug in the microdialysate that has passed through the probe. In this way, the dose of morphine delivered during a 30-min period perfusion of a microdialysis probe in the PAG was determined [56].

6. Microdialysis in various CNS-regions—technical aspects

6.1. Microdialysis in the spinal cord

To our knowledge, three different methods for spinal microdialysis have been presented. First, a microdialysis fiber introduced transversally through the rat dorsal spinal cord [28,72], which enables experiments on awake animals [13,34,63,73,74]. Second, microdialysis with a conventional concentric microdialysis probe introduced through the dorsal surface of the spinal cord of decerebrated cats [52] and anaesthetized rats [12,17], which has been used for monitoring of neuropeptides and amino acids [11,12,75,76]. In order to increase the volume of dorsal horn tissue sampled by this approach, the

Table 2
Formalin test and in vivo microdialysis

Region	Neurotransmitter	Manipulation/drugs	Refs.
Dorsal horn– transverse probe	Glu ↑, PGE2 ↑, NO ↑ PGE2 ↑	Acetaminophen: PGE2↓ PGE2↓ COX-1 inhibitor: PGE2↓ COX-2 inhibitor: PGE2 ↔	[103,104] [63,105,106]
	Glu ↑, Asp ↑	Formalin+TTX: Asp ↓ Formalin+TTX: Glu ↔ Veratridine: Glu ↑ Veratridine+TTX: Glu↔	[28]
	Asp ↑, Glu ↑, Asn ↑, Gly↑, Tau ↑	SP(1–11): Asp ↑, Glu ↑, Asn ↑, Gly ↑, Tau ↑ SP(5–11): Asp ↑, Glu ↑, Asn ↑, Gly ↑, Tau ↑ SP(1–7): Glu ↓, Asn ↓, Gly↓, Tau ↓ Neonatal capsaicin: PGE2↓, Glu ↔, Asp ↔, Tau ↔	[33] [107]
Intrathecal loop dialysis	PGE2 ↑ Glu ↑, Asp ↑, Tau ↑ PGE2 ↑ Asp ↑, Glu ↑ Glu ↑ Glu ↑, Asp ↑, Tau ↑, Gly↑, Cit ↑, PGE2 ↑ Ser ↔, Asn ↔, Gln↔ Glu ↑, Asp ↑, Gly ↑, Tau↑, Ser ↑, PGE2 ↑	S-ibuprofen, COX-2 inhibitors: PGE2↓, Glu ↓, Asp ↓ Remifentanyl: Glu ↓ Morphine (naloxone reversible): Glu ↓, Asp ↓, Tau ↓, Gly ↓, Cit ↓, PGE2 ↓	[51,108] [109] [62,79]
	Formaline 5%: Formaline 10%: Substance P ↑ PGE2 ↑	Evoked release blocked by alpha-trinositol (nitrite/nitrate) ↑; Glu ↑ (nitrite/nitrate) ↔; Glu ↔	[110] [111]
		Diabetes ↓ Diabetes ↑, COX-inhibitor ↓	[112] [113]
		Formalin+TTX: Glu ↔, Asp↔, Arg ↔ Formalin+calcium free: Glu ↔, Asp↔, Arg ↔	[39]
		GABA ↓, Glu ↓ Gln ↔, Gly ↓	Naloxone reversible Basal level TTX: Glutamine ↔ TTX: Glycine ↓ Naloxone: no effect
Rostral ventromedial medulla (RVM)	Anandamide ↑ Serotonin (5-HT) ↑	Basal 5-HT level: TTX: 5-HT ↓ Calcium free: 5-HT↓ Citalopram: 5-HT ↑ Pargyline: 5-HT ↑ Amphetamine: 5-HT ↑ 8-OH-2-(di- <i>n</i> -propyl-amino)tetralin: 5-HT ↓	[65] [10]
	Accumbens	Met-enkephalin-LI ↑, CCK-LI ↑	Formalin+ Naloxone: CCK-LI ↔
Arcuate nucleus	Beta-endorphin ↑		[61]
Ventral posterolateral thalamus (VPL)	Glu ↑, Asp↑, Arg ↑	Formalin+TTX: Glu ↔, Asp↔, Arg ↔ Formalin+calcium free: Glu ↔, Asp↔, Arg ↔	[83]

TTX: tetrodotoxin; Glu: Glutamate; Asp: Aspartate; Gln: Glutamine; Asn: Asparagine; Ser: Serine; Cit: Citrulline; Arg: Arginine; Thr: Threonine; PGE2: Prostaglandin E2; ↑=increased release, ↓=decreased release, ↔ no change or blocked release.

Table 3
Acute and sustained peripheral inflammation and microdialysis

Inflammation	Region/anesthesia	Neurotransmitter	Manipulation/drugs	Ref.
Carrageenan hind paw inj.	Dorsal horn	CCK-LI ↔	Morphine + Carrageenan:	[114]
	Halothane		CCK-LI ↔	
	Anesthesia, rat		Morphine–Carrageenan: CCK-LI ↑	
	Dorsal horn	PGE2 ↑		[115]
	Awake rat			
	Dorsal horn	5-HT↑, 5-HIAA↑	Carrageenan + Naloxone (icv):	[116]
Kaolin/ Carrageenan Knee joint	Awake rat		5-HT↔, 5-HIAA↔	
	Dorsal horn		Carrageenan + Bicuculline:	
	Awake rat	5-HT↑, 5-HIAA↑	5-HT↑, 5-HIAA↑	[116]
	Dorsal horn	5-HT↑, 5-HIAA↑	Carrageenan + Bicuculline:	
Kaolin/ Carrageenan Knee joint	Awake rat	5-HT↑, 5-HIAA↑	5-HT↑↑, 5-HIAA↑↑	[117]
	Dorsal horn	Early phase	EaP + AP7 or CNQX:	[74]
	Awake rat	(EaP): Glu ↑, Asp ↑, Gln ↑	Glu ↔, Asp ↔, Gln ↔	
		Late phase (LaP):	LaP + AP7, CNQX or SP-	
	Dorsal horn	Glu ↑, Asp ↑, Gln ↑	antagonist: Glu ↔, Asp ↔, Gln ↔	
	Awake rat	Glu ↑, Asp ↑, Gln↑, Ser↑, Gly ↑	LaP + Bicuculline: Glu ↔, Asp↔	[117]
			LaP + GABA-B antagonist	
	Dorsal horn	Glu ↑, Asp ↑, Gly↑	(CGP35348): Glu ↑, Asp ↑, Gln↑	[118]
	Anest. Monkey	Ser ↑, Gln ↓, Tau ↑		
	Intrathecal loop	Glu ↑, Asp ↑, Cit ↑		[119]
dialysis, awake rat	PGE2 ↑			
Intrathecal loop	PGE2 ↑,	+ Capsaicin: PGE2 ↑↑	[120]	
dialysis, awake rat	6-ketoPGF1α ↔	+ Capsaicin + S-ibuprofen: PGE2 ↔		
		+ SP: PGE2 ↑		
Complete Freund's adjuvans (CFA) hindpaw inj.	PAG, awake rat	GABA ↓, Met-enkephalin ↑, Neurotensin ↑		[26,59]
Turpentine induced oral inflammation	Trigeminal ganglion, awake guinea pig	SP-LI ↑	+ Calcium free: SP-LI ↓	[24]

Glu: Glutamate; Asp: Aspartate; Gln: Glutamine; Asn: Asparagine; Ser: Serine; Cit: Citrulline; Arg: Arginine; Thr: Threonine; PGE2: Prostaglandin E2; 5-HIAA = 5-hydroxyindoleacetic acid; ↑ = increased release, ↑↑ = potentiated increased release, ↓ = decreased release, ↔ = no change or blocked release. + pain stimulus in combination with other agents.

dialysis probe can be inserted at an oblique angle of 45° from the horizontal plane in the rostrocaudal direction [77]. Using an angle of only 12°, chronic microdialysis in the spinal cord with this approach has been performed in awake animals [78].

A third method, intrathecal microdialysis, involves the introduction of a loop of microdialysis membrane introduced through the atlanto-occipital membrane and then advanced to the lumbar level [79]. The surgical procedures are essentially identical to the insertion of an intrathecal catheter as originally described by [80].

6.2. Brain microdialysis

For further technical details on brain microdialysis we refer to previous reviews [1,2,5].

Custom-made as well as commercially available microdialysis probes have been used. Some researchers implant the microdialysis probe in one step, whereas others first implant a guide cannulae (24–48 h prior to the experiments). This latter approach reduces the risk of scarring around the probe and bypasses the need to anesthetize the animal just prior to the collection of samples. For details on the

Table 4
Nerve injury and microdialysis

Nerve injury	Region/anesthesia	Neurotransmitter	Manipulation/drugs	Ref.
Axotomy of the sciatic nerve	Dorsal horn, rat, halothane	CCK-LI	Potassium: CCK-LI ↔ Potassium+CCK-B antagonist (CI988): CCK-LI ↑	[11]
		SP-LI	Potassium: SP-LI ↓	[18]
		CCK-LI	Potassium: 1–7 days post axo.: CCK-LI ↑ 2 weeks–2 months post axo.: CCK-LI ↔	[75]
Spinal nerve ligation L5, L6 Partial sciatic nerve ligation Seltzer et al., 1990 [123]	Cingulate cortex, rat, awake	CCK-LI	Potassium: CCK-LI ↑	[91]
	Intrathecal dialysis rat, awake	Prostaglandin	+ brushing: PGE2 ↑	[121]
	Dorsal horn rat, halothane	Allodynia: GABA ↓ No allodynia: GABA ↔ SP-LI	Spinal cord stimulation (SCS): GABA ↑, Glu ↓, Asp ↓	[76,77]
	Intrathecal dialysis rat, awake	Glu, Asp	Potassium: SP-LI ↑ + SP: Glu ↑, Asp ↑ Neonatal capsaicin+SP: Glu ↔, Asp ↔	[18] [122]

Glu: Glutamate; Asp: Aspartate; PGE2: Prostaglandin E2, SP-LI: Substance P like immunoreactivity, CCK-LI: CCK-like immunoreactivity. ↑=increased release, ↓=decreased release, ↔ no change or blocked release. +nerve lesion in combination with other agents.

impact of a brain microdialysis probe on the surrounding tissue see [81]. If a guide cannula is in place, the microdialysis probe can be inserted into the brain of the awake animal with minimal restraint.

Despite the prefix 'micro', the microdialysis probe is quite large (most common 200–500 μm diameter and a length of 2 mm), relative to many rat brain regions of interest for pain research. Certain medullary areas may not be investigated by standard microdialysis, since vital groups of neurons, such as respiratory neurons of the nucleus tractus solitarius, may be destroyed by the microdialysis probe, leading to death.

An interesting approach to limit the sampling area of brain microdialysis is the use of probes with a membrane on one side only [27].

Brain regions of importance for modulation and transmission of nociceptive signals that have been studied by microdialysis include: thalamic nuclei, receiving direct spinal nociceptive input; areas considered to be of importance for the affective component of pain perception, such as the nucleus accumbens and the anterior cingulate cortex; mid-

brain and brainstem areas coordinating descending modulation of nociceptive signaling, the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM).

6.2.1. Thalamic nuclei

Morphine and riluzole (a drug that blocks glutamate transmission) at doses which blocked hyperalgesia after carrageenan injection into the hindpaw decreased the glutamate level in the ventral posterolateral nucleus of the thalamus in the presence and absence of a painful stimulation (paw pressure). In this region the aspartate, citrulline and arginine levels were decreased by riluzole, but not morphine [82].

Subcutaneous formalin (5%, 50 microl) injection in the right hind paw of the rat caused an increase of arginine, glutamate and aspartate in the ventral posterolateral thalamic nucleus [83].

Systemic administration of tiagabine, a GABA uptake inhibitor, at a dose that significantly increased the paw pressure nociceptive threshold, increased nearly two-fold the extracellular gamma-amino-

butyric acid (GABA) levels in the medial thalamus in rats [84]. Tight ligation of the L5, L6 spinal nerve of the rat, an animal model of neuropathic pain, leads to decreased 5-HT content in dialysates of the contralateral ventrobasal thalamus compared with SHAM rats. The noradrenaline level in this region was not affected by the nerve lesion [85].

6.2.2. *Nucleus accumbens*

Injection of 50 microl of 5% formalin into the forepaw, which induces a pain-related behavior, also resulted in an immediate and sustained increase of met-enkephalin-like material (+27%) and CCK-LI (200–250% of the basal level) within the nucleus accumbens. Naloxone (1.5 mg/kg i.p., 10 min prior to formalin injection) prevented the increase of CCK-LI [86].

Following chronic morphine administration using the conditioning place preference paradigm, increased enkephalin levels were observed in the nucleus accumbens of rats placed in the drug-associated compartment. Decreased enkephalin levels were found in the saline-paired side [87].

Based on previous findings demonstrating blockade of pain-induced analgesia by injection of a dopamine antagonist into the nucleus accumbens, Schmidt et al. evaluated the effects of s.c. capsaicin administration on accumbal dopamine concentrations. They found that the noxious stimulation by capsaicin increased dialysate dopamine, indicating that dopamine release in the nucleus accumbens parallels antinociception [88].

6.2.3. *Anterior cingulate cortex (ACC)*

The anterior cingulate cortex is a brain area, which is activated by painful stimulation as demonstrated by positron-emission-tomography studies in man [89,90]. In a recent microdialysis study we have demonstrated increased potassium induced release of cholecystinin in the ACC following complete unilateral transection of the sciatic nerve [91], a model of phantom limb pain or anaesthesia dolorosa [92].

6.2.4. *Periaqueductal gray (PAG)*

A brain area that has attracted large interest for pain research is the PAG. Peripheral inflammation induced by injection of Complete Freund's Adjuvant

(CFA) into the hind paw significantly decreased the GABA release [26] and increased neurotensin and Met-enkephalin release in the PAG [59].

A formalin injection in the hindpaw of the rat increased glutamate, arginine and aspartate concentration in PAG dialysates. Handling, pinching or saline injection in the hind paw did not increase glutamate, showing that this neurochemical phenomenon is related to painful and persistent noxious stimulation [39]. Systemic morphine administration has also been shown to decrease the veratridine induced release of GABA in the lateral, but not the medial part of the PAG of the rat [27]. Local administration of morphine into the PAG also decreased the basal release of GABA in the PAG [56]. In control rats systemic administration of morphine, but not saline induced a significant release of histamine in the PAG [93].

6.2.5. *The rostral ventral medulla (RVM)*

This area, which receives input from the PAG sends projections to the spinal cord. The excitability of dorsal horn neurons is controlled in part by RVM neurons; for review see [94,95]. Taylor and Basbaum found that intraplantar formalin increased dialysate 5HT in RVM, suggesting that this neurotransmitter contributes to nociceptive modulation by regulating the outflow of the rostral ventromedial medulla neurons [10].

7. Microdialysis in pain models

Microdialysis has become the method of choice for estimating neurotransmitter release in a number of animal pain models. We have assembled many of these studies into the following groups: acute mechanical, chemical and electrical nociceptive stimuli (Table 1), the formalin test (Table 2), tests of acute and prolonged peripheral inflammation (Table 3) and models of neuropathic pain (Table 4). An immediate observation of this compilation is that most studies involve models of acute inflammatory pain, while only rather few studies have been performed in models of long lasting inflammatory or neuropathic pain.

In clinical settings acute pain can be handled reasonably well with the available analgesic drugs,

while the pain associated with some forms of peripheral nerve injury, diabetic neuropathy, arthritis, stroke and cancer are often resistant to the currently available treatment modalities. Therefore, we suggest that future microdialysis experiments should focus on neurotransmitter and neuromodulator release during chronic pain states. One example of this approach is the observation of decreased GABA levels in the dorsal horn in rats with allodynia after a partial nerve lesion. Spinal cord stimulation (SCS), which reversed the allodynia also increased the extracellular GABA level [77]. These findings suggested that spinal administration of GABA decreases allodynia and a combination of subeffective doses of GABA agonists with SCS were shown to potentiate the antiallodynic effect [96]. Similar response was also seen in patients (Meyerson, personal communication). Thus, comparison of animals that develop signs of neuropathic pain with animals that do not develop these signs may provide the clues for the treatment of these conditions.

8. Conclusion

In vivo microdialysis is an established method for monitoring the extracellular level of neurotransmitters in the CNS. Several regions of interest for pain transmission and pain modulation have been studied with regard to the release of amino acids, monoamines, neuropeptides and prostanoids in mostly acute nociceptive pain models. Animal models of chronic pain conditions, often resistant to available treatment have only been studied to a limited extent. A dialog between the clinician and the basic pain scientist may result in the design of microdialysis studies of pain models, which more closely resemble the clinical pain conditions and thus may produce outcomes with more direct therapeutic implications.

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