Pharmacology Biochemistry & Behavior, Vol 34, pp 107–112 © Pergamon Press plc, 1989 Printed in the U S A 0091-3057/89 $3 00 + 00

Regional In Vivo Superfusion of the Spinal Cord and KCl-Induced Amino Acid Release[1]

DAVID B MASTERS,*[2,3] FRANK JORDAN† AND BARRY R. KOMISARUK*

**Institute of Animal Behavior, Rutgers, The State University of New Jersey, 101 Warren Street, Newark, NJ 07102*
†Department of Chemistry, Olson Laboratories, Newark, NJ 07102

Received 28 June 1989

MASTERS, D B , F JORDAN AND B R KOMISARUK *Regional in vivo superfusion of the spinal cord and KCl-induced amino acid release* PHARMACOL BIOCHEM BEHAV **34**(1) 107–112, 1989 —In vivo push-pull superfusion was used to sample the regional release of amino acids into spinal superfusates of urethane-anesthetized rats By collecting superfusates from the intrathecal space surrounding the sacral-lower thoracic spinal cord, it was possible to achieve a stable release of amino acids in one- and five-minute superfusate fractions Introducing the depolarizing agent, potassium chloride (KCl) (40 mM), into the superfusion medium significantly increased GLU, GLY, and TAU concentrations in superfusates compared to pre-KCl values The findings that these three amino acids were the only ones (out of 20) that showed a significant increase in response to KCl administration, suggest that they mediate neurotransmission in this region of the spinal cord Amino acid concentrations were determined in spinal superfusates by high performance liquid chromatography (HPLC), utilizing an automated ortho-phthaldialdehyde precolumn derivatization system The regional superfusion system described in this paper provides a technique for measuring KCl-produced release of neurochemicals that may mediate neurotransmission in delimited spinal regions

Push-pull superfusion Regional superfusion Amino acids Spinal cord Sensory stimulation
Potassium stimulation Intrathecal space

THE objective of this study was to measure the release of specific amino acids in response to chemical depolarization from a delimited region of the spinal cord We ascertained whether amino acids are released in response to potassium chloride (KCl), which has been shown to release neurotransmitters when added to the spinal superfusion medium (16,17)

Early investigations of amino acid systems in the central nervous system (CNS) utilized an in vivo subarachnoid superfusion system for measuring glycine transport from spinal fluid (2) Shortly thereafter, spinal superfusion was used for the application of narcotics (15) and for measuring the release of endogenous substances from spinal cord tissue in response to chemical and sensory stimulation (16,17) Using these techniques, a catheter was implanted down through the intrathecal space of the cisterna magna so that its tip expelled perfusion fluid at the lumbar level. In push-pull superfusions a second catheter was also used to collect the superfusate from the cisterna magna opening These "full length" spinal superfusion procedures collected neurochemicals from the entire intrathecal space surrounding the spinal cord and part of the cauda equina However, because neurochemicals are released from synaptic terminals within the spinal cord, they must pass through spinal tissue before diffusing into the superfusion medium This barrier makes their measurement susceptible to the effects of dilution, uptake and transport systems, and/or enzymatic breakdown processes Moreover, the specific spinal site of release cannot be ascertained using these procedures Therefore, we developed a method for localizing the release and collection of amino acids in sacral-lower thoracic spinal cord superfusates in response to chemical stimulation The data presented here demonstrate a significant and specific release of endogenous amino acids in response to the depolarizing agent, KCl (13)

METHOD

Subjects

Intact female Sprague-Dawley rats were used, weighing 310–370 g, group-housed at 23°C and maintained on a reverse light cycle (10 hr dark). Food and water were supplied ad lib Rats were anesthetized with 1 5 g/kg urethane hydrochloride one hour prior to implantation of two calibrated catheters into the intrathecal

[1]Preliminary results of this paper were presented at the 18th Annual Meeting of the Society for Neuroscience (November 1988, Toronto)

[2]D B Masters was a recipient of an Omnitech Travel Fellowship (Honorable Mention) to the Society for Neuroscience Meeting, 1988 This paper is based on the abstract submitted for the award The award winning papers are published together in this issue

[3]Requests for reprints should be addressed to D B Masters, Harvard Medical School, The Children's Hospital, Department of Anesthesia, 300 Longwood Drive, Boston, MA 02115

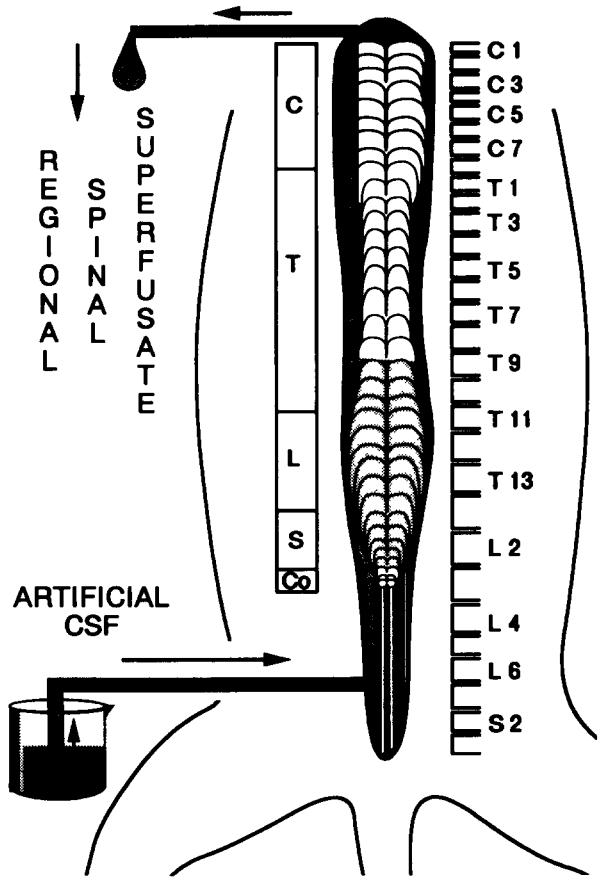

FIG 1 Schematic diagram of regional in vivo push-pull superfusion of the intrathecal space surrounding the sacral-lower thoracic spinal cord (T10–S4) The labels on the right side identify the vertebrae and the labels on the left side identify the segmental levels of the spinal cord (center) C = cervical, T = thoracic, L = lumbar, S = sacral, and Co = coccygeal

space (Clay Adams PE-10 tubing, Fisher Chemical Co) Immediately after all superfusate fractions were collected the anesthetized animal was euthanised with an overdose of urethane

Catheterization

Using a modified push-pull superfusion procedure that was adapted from previously reported techniques (9,15), artificial cerebrospinal fluid (CSF) was superfused out of one catheter at the 4th sacral segment (push) and into a second catheter at the 10th thoracic segment (pull) of the spinal cord in order to sample amino acid release from the intermediate spinal segments (Fig 1) Prior to surgery, the animal was oriented vertically in a stereotaxic headholder (Trent-Wells Co) The hindpaws were allowed to reach the bench-top to support the rat while its head was flexed forward and held at a level position This posture provided for easier catheter insertion and thereby decreased the possibility of causing damage to the cord during intrathecal implantation The intake catheter was inserted into the intrathecal space through a hole in the atlanto-occipital membrane (made by a 24-gauge needle) and positioned to pull fluid from its tip 6 0 cm caudally from the cisterna magna at the 10th thoracic segment of the spinal cord (15) After careful removal from the stereotaxic holder, the animal's lower abdomen was placed on a cushion and positioned to mimic a lordotic posture Next, a dorsal midline incision was made to expose the 6th lumbar and 1st sacral vertebrae (9) Retraction of muscle and some removal of tissue between the vertebral spinous processes exposed the vertebral lamina and foramen which contains the dura-covered spinal cord Without damaging any blood vessels, a 24-gauge needle was used to pierce the dura mater The output catheter (kept parallel to the rachis) was inserted through this hole in the dura membrane, 2 5 cm rostral to its insertion point, so that its output tip was at the caudal end of the sacral spinal cord Both catheters were filled and capped with artificial CSF just prior to implantation The surgical areas were kept moist with wet cotton gauze after surgery and during superfusate collection

Regional Push-Pull Superfusion

The catheters were attached to a peristaltic pump (Model 203, Scientific Industries Inc) precalibrated to achieve 100 μl/min flow rate Push-pull superfusion was delayed 20–30 min after completion of surgery Artificial CSF (7 46 g NaCl, 0 19 g KCl, 0 14 g $CaCl_2$, 0 19 g $MgCl_2$, 0 18 g H_3PO_4, 120 mg BSA, 300 mg bacitracin/liter of HPLC grade water), maintained at 7 4 pH with O_2 CO_2 gas (95 5 ratio) gently bubbled into it, was superfused over the sacral-lower thoracic spinal cord In some cases, initial efforts were needed to position the animal to allow for a free flow of fluid through the catheters Once a continuous flow was established, a fraction collector (ISCO) was used to collect five-minute superfusate fractions into microcentrifuge tubes (Eppendorff 1 5 ml) containing 10 μl of 0 1% perchloric acid to denature proteins for enzyme inhibition After 30 min of baseline collection, KCl administration was achieved by increasing the KCl in the superfusion medium from 3 4 to 40 0 mM Each five-minute superfusate fraction collected was immediately put on ice before subsequent centrifugation (12,000 × g, at 0°C for 3 min) The supernatant was removed by pipette and stored at −80°C prior to amino acid analysis In two animals, after collection of amino acid superfusates was completed, Evans Blue dye was added to the superfusion medium to examine the flow of fluid through the intrathecal space Autopsy revealed that the superfused dye surrounded the spinal cord and remained confined to the intrathecal space between the catheter tips All superfusions were terminated and discarded if blood appeared in the superfusate

Superfusion Recovery Rate

In order to correct basal amino acid values from superfusion dilution effects, a recovery factor was used to convert the amino acid values to their undiluted basal concentrations (Table 1) This recovery factor was determined by extrapolating a previously reported value (35% of whole CSF) from a push-pull superfusion study that utilized a "full length" preparation at 24 5 μl/min flow rate (4) In comparison, the regionally superfused amino acids described here were collected from an approximately ⅓ smaller volume and at a 4-fold faster flow rate than the "full length" technique Therefore, a lower rate of recovery from greater dilution was expected Indeed, after estimating the intrathecal (IT) volume (0 15 cc and 0 42 cc for "regional" and "full length" techniques, respectively) based upon average spinal dimensions (3 6 mm = spinal cord diameter, 0 25 × 14 0 mm = IT width × length), a general amino acid recovery rate of 20 4% of whole CSF was estimated for the first ml (two five-minute fractions) of regional push-pull superfusate The following two equations were used to estimate this recovery rate (subscripts R and F refer to "regional" and "full length" superfusion techniques respectively)

$$\text{flow rate}_F \times 10 \text{ min} + \text{IT vol}_F = T_e(\text{flow rate}_R \times 10 \text{ min} + \text{IT vol}_R) \quad (1)$$

$$\text{recovery rate}_R = \text{recovery rate}_F \times T_e \quad (2)$$

It was assumed that the extrapolation factor (T_e) corrected the basal levels of amino acids for a linear dilution effect

HPLC Instrumentation

The chromatographic apparatus comprised two Beckman pumps (114M), a Beckman Gradient Controller (421), and a Spectra-Physics integrator (SP4270) A Gilson Automatic Sample Dilutor (231) controlled reagent and sample mixing for precolumn derivatization and column injection. A Phenomenex (3 μ, C18) ultramex analytical column (4 6 × 150 mm) preceded by a similar guard column (30 mm) was used for amino acid separation. The column effluent was monitored by a LDC/Milton Roy Fluorometer-III (1311) with factory standard 370 nm excitation and 418–700 nm emission filters

Reagents

HPLC grade water, methanol, and tetrahydrofuran (THF) were Baker Chemical Co products purchased from ACE Chemical Co Ortho-phthaldialdehyde (OPA), fluoraldehyde reagent diluent, Brij-35, 2-mercaptoethanol, and amino acid standards were obtained from Pierce Chemical Co Ultra-high purity sodium acetate was purchased from Aldrich Chemical Co All other chemicals were reagent grade

Derivatization and Chromatography

The derivatizing reagent was prepared in an opaque test tube and stored at 4°C a maximum of 10 days First, 10 mg of OPA was dissolved in 200 μl of methanol and then 9 75 ml fluoraldehyde diluent, 0 03 ml Brij-35 (30%), and 0 02 ml 2-mercaptoethanol were added and mixed by vortex for 30 sec Automated precolumn derivatization was accomplished by taking up 20 μl of OPA reagent and 40 μl of superfusate or standard into the Dilutor chamber prior to three mixing cycles in a third vial containing 20 μl of internal standard (2-aminoisobutyric acid) Immediately following the last mixing cycle, forty μl of this mixture was pushed through a 20 μl injection loop and injected onto the column Therefore, amino acid derivatization proceeded for 60 sec in total before being terminated by the initiation of the chromatography process All amino acid standards were dissolved in fluoraldehyde diluent to maximize and stabilize the OPA reaction

The mobile phase consisted of two solvents that were mixed together in a gradient of increasing organic concentration by two differential pumping rates (constant flow rate 0 9 ml/min) Solvent A consisted of 0 10 M sodium acetate, 10% (v/v) methanol, and 0 5% (v/v) THF, adjusted to pH 7 0 with glacial acetic acid Solvent B contained a mixture of methanol water in a 90 10 ratio Both solvents were passed through a 0 46 μm nylon filter, degassed under vacuum during sonication (or magnetic stirring) before being sparged and blanketed with helium gas Each gradient program ended with a 10-min hold at 95% solvent B followed by a 12-min reequilibration with 100% solvent A (see Fig 2B)

HPLC Standard Analyses

Since an internal standard method of HPLC analysis was used in addition to an external method, variations in amino acid quantification due to the OPA reaction were corrected Figures 3 and 4 show that the internal standard, 2-aminoisobutyric acid (ABA), did not significantly vary across or between analyzed superfusate fractions In fact, if ABA values did not remain consistent, the HPLC system was corrected and the samples were reanalyzed Therefore, OPA derivatization was very reproducible and required little normalization between samples

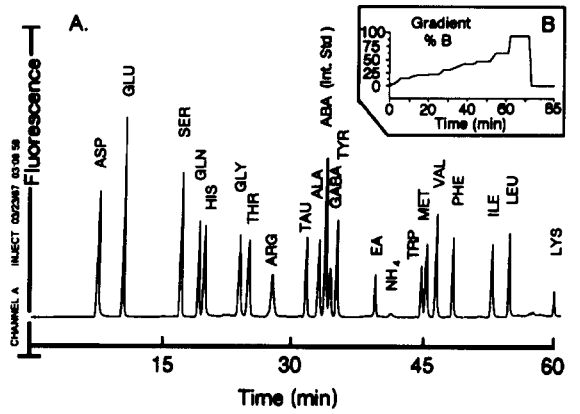

FIG 2 (A) HPLC separation of ortho-phthaldialdehyde-derivatized amino acid standards (17 0 picomoles/20 μl) with the internal standard 2-aminoisobutyric acid (ABA) at 34 0 picomoles/20 μl (B) Gradient profile of solvent B percentage during the two-solvent chromatographic process TAU = taurine, NH_4 = ammonia, EA = ethanolamine

In addition, repeated analysis (8 trials) of a standard solution (20 amino acids), with internal methods employed, revealed a mean relative standard deviation below 3 7% (SEM = 0 6%) Using a one-way ANOVA with repeated measures on these data, the between variance mean square (MS) component was divided by the total variance (sum of between and within variance MS components) to give a Falconer's repeatability score of r = 90 (3) This score predicted that the addition of a second or third HPLC value would increase the accuracy of these measurements by only 5 0 or 7 5%, respectively Therefore, each superfusate sample was measured once Standards were run at the beginning and end of every sample queue and at least after every 5th superfusate (Fig 2A)

RESULTS

Basal Amino Acid Levels

Basal amino acid concentrations that were determined from the first two superfusate fractions are presented in Table 1 These values were corrected for superfusion dilution effects, based on a recovery rate from a previously reported in vivo study (see the Method section) Gamma-aminobutyric acid (GABA) superfusate concentrations were below detectable limits (0.05 μM) for the first two fractions collected in four of the five animals tested.

Pre-KCl Superfusates

The pre-KCl release of amino acids into regional spinal superfusates is presented in absolute uncorrected concentrations (Fig 3) and in percent change from control values (Fig 4) ANOVA with repeated measures (two within-variable design) compared amino acid concentrations in the two fractions collected just prior to KCl administration (fractions No 5 and No 6) This test found that various amino acids were present in concentrations that differed significantly from each other, $F(18,72) = 44\ 30$, $p < 0.0001$ Moreover, levels of amino acids did not differ significantly between successive fractions 5 and 6, $F(1,4) = 0\ 239$, $p > 0\ 65$, for the individual amino acid, $F(18,72) = 0\ 694$, $p > 0\ 80$. In two control animals (data not shown), after a 20-min initial equilibration period, local spinal superfusion (over 90 min) showed

TABLE 1

AMINO ACID CONCENTRATION IN SPINAL SUPERFUSATE

Amino Acid	Basal Concentrations (μM) Corrected	Uncorrected
ASP	5 20 ± 2 30	1 06 ± 0 47
GLU	12 71 ± 3 97	2 59 ± 0 81
SER	22 57 ± 4 95	4 60 ± 1 01
GLN	104 72 ± 10 21	21 34 ± 2 08
HIS	8 49 ± 1 86	1 73 ± 0 38
GLY	16 58 ± 2 45	3 38 ± 0 50
THR	25 85 ± 4 91	5 28 ± 1 07
ARG	16 63 ± 2 55	3 39 ± 0 52
TAU	18 76 ± 4 17	3 82 ± 0 85
ALA	26 11 ± 2 25	5 32 ± 0 46
GABA	<0 25 —	<0 05 —
TYR	3 39 ± 0 59	0 69 ± 0 12
EA	9 72 ± 2 65	1 98 ± 0 54
TRP	2 01 ± 0 48	0 41 ± 0 10
MET	3 04 ± 0 59	0 62 ± 0 12
VAL	8 94 ± 1 72	1 82 ± 0 35
PHE	4 12 ± 0 94	0 84 ± 0 19
ILE	4 42 ± 0 74	0 90 ± 0 15
LEU	7 45 ± 1 52	1 52 ± 0 31
LYS	52 66 ± 12 18	10 73 ± 2 48

Basal amino acid concentrations represent mean ± SEM values from the first two superfusates in five animals Superfusate amino acid values were corrected for dilution (see the Method section)

a steady slow decrease in amino acid recovery during resting conditions over successive superfusates (less than 5% average decline between fractions)

KCl Administration

KCl administration increased the release of several amino acids from the spinal cord into the superfusates above pre-KCl concentrations (Figs 3 and 4) Statistical analysis of amino acid concentrations compared the five-minute fractions immediately prior to KCl administration with the maximum values obtained during KCl administration On the basis of ANOVA with repeated measures (two within-variable design), the concentrations of various amino acids differed significantly among each other, $F(18,72) = 27\ 07$, $p<0\ 0001$, and these concentrations changed significantly between pre-KCl and KCl superfusates, $F(1,4) = 9\ 73$, $p<0\ 036$ This test also showed a significant interaction term, $F(18,72) = 2\ 075$, $p<0\ 016$, indicating that not all individual amino acids responded to KCl administration in the same way The simple effects analysis of this interaction showed that only GLU, $F(1,4) = 16\ 75$, $p<0\ 015$, GLY, $F(1,4) = 9\ 71$, $p<0\ 036$, and TAU, $F(1,4) = 14\ 205$, $p<0.020$, concentrations were significantly increased above pre-KCl levels during KCl administration (Fig 4) GABA pre-KCl and KCl concentrations were determined in two animals

DISCUSSION

The present study has shown that in vivo "regional" superfusion coupled to a sensitive HPLC-fluorescence detection system is an effective technique for measuring the release of endogenous amino acids into the extracellular fluid of the spinal cord By using anesthetized animals, it was possible to collect spinal superfusates that rapidly achieved stable amino acid concentrations without

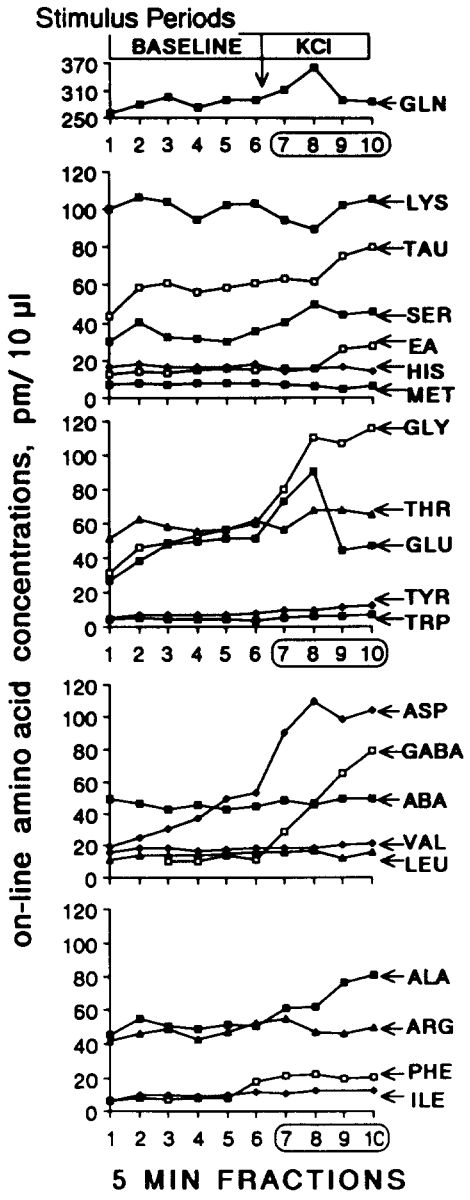

FIG 3 Time course of release of individual amino acids into regional spinal superfusates before (fractions 1–6) and during KCl administration (fractions 7–10) in one animal Five separate graph panels were used in this figure to enhance graphing clarity, no special grouping is implied The internal standard, ABA, was added to superfusates just prior to amino acid analysis

catheter displacement produced by locomotor activity Using this system, we found a significantly increased release of GLU, GLY, and TAU from the sacral-lower thoracic region of the spinal cord in response to KCl administration KCl has been shown to release amino acids from neurons as well as glia (13) Consequently, the present study does not differentiate between these two sources of amino acids Furthermore, the Ca^{2+}-dependence of the KCl-evoked release of amino acids can not be addressed by this regional superfusion system, because to date no technique exists to remove all Ca^{2+} from the spinal cord in situ Although several investigations have demonstrated the evoked release of ASP, GLU, GABA, GLY and TAU from in vitro preparations (brain and spinal cord) to be largely Ca^{2+}-dependent (1, 11, 12), it is not

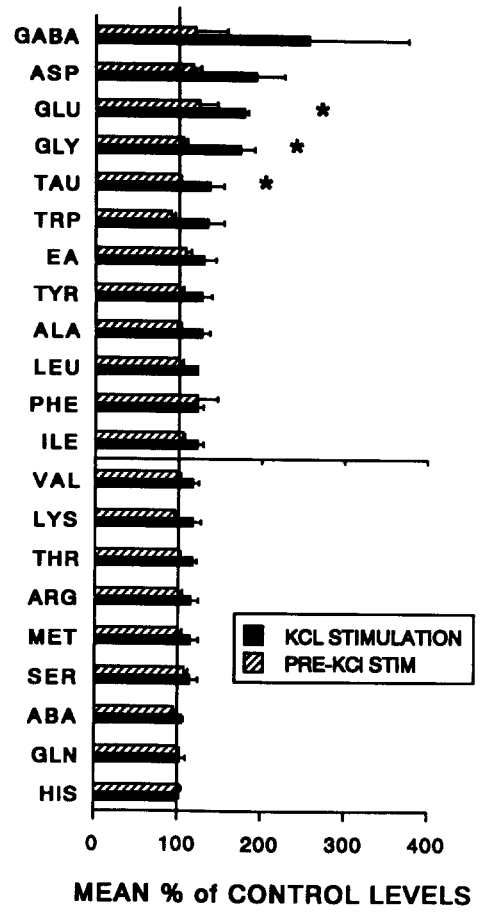

FIG 4 Increasing KCl concentration to 40 mM enhanced the release of amino acids into superfusates collected from the sacral-lower thoracic spinal cord in anesthetized animals (n = 5) This figure shows the release of amino acids during five-minute fractions as a mean percentage ± SEM of the period before pre-KCl- and KCl-administered fractions, respectively Since the fraction containing the largest KCl-induced release of individual amino acids varied between superfusates, the maximum KCl value was used An asterisk indicates a significant difference between these values (see the Results section) GABA results are based on two animals

clear that this release is of vesicular origin (8) However, a specific sensory stimulus (vaginocervical mechanostimulation) has been found (10) to produce a profile of amino acid release that is similar to that produced by KCl in the present study Therefore, the amino acid release produced by KCl in the present study presumably results, at least in part, from neuronal stimulation

The corrected mean basal concentrations of amino acids determined in this study are in agreement with whole CSF values from male rats (6,7) Although contamination of CSF by blood can lead to variable and erroneous measurements of amino acid concentrations (7), our data show a stable rate of release of amino acids into spinal superfusates during resting conditions over the time course of this experiment (slow decline over 90 min) Even if blood were present but undetected, it is unlikely that it would have been present in some consistent relationship with the KCl Therefore, there is no reason to believe that the amino acid levels were affected by undetected blood in the superfusate in any consistent way Furthermore, in a recent microdialysis study that measured basal concentrations of extracellular amino acids from the spinal cord of awake male rats of the same strain, it was found that all reported midlumbar dorsal horn concentrations and their standard deviations (ASP, GLU, GLY, and TAU) were nearly identical to the corrected basal values we obtained from our regional superfusates (14). Therefore, it would appear that these very different procedures collect amino acids from the same or similar compartment pools

In comparison, the present regional push-pull superfusion method has a number of advantages over other sampling techniques in the collection of neurochemicals released from the spinal cord For example, microdialysis techniques produce damage to the spinal cord, whereas regional push-pull superfusion is noninvasive to the spinal cord In addition, regional push-pull superfusion straddles a selected region of the spinal cord, whereas push-pull cannulation or traditional push-pull superfusion methods are restricted to either collecting superfusate from a minute spinal region or the entire length of spinal cord, respectively Consequently, regional push-pull superfusion provides a technique for measuring the release of neurochemicals from specified regions of the spinal cord without invading the spinal tissue under investigation

GABA was not detected in many superfusates According to human CSF studies, approximately 2% of the total GABA is in the free form, the rest is mostly a lactam, 2-pyrrolidinone, or conjugated to histidine as the dipeptide, homocarnosine (5) Since we only measured the free form, it is not surprising that basal GABA concentrations were below our detection limits

With existing HPLC-superfusion sensitivity, it is possible to collect an adequate sample in a brief period This can permit ascertaining the effect of a brief stimulus (chemical or sensory) on amino acid release from a delimited region of the spinal cord In preliminary experiments, it was possible to measure amino acids in one-minute superfusate fractions of the sacral-lower thoracic spinal cord (at a superfusion rate of 100 μl/min) and achieve stable concentrations in successive samples This is an advantage of "regional" over "full length" spinal superfusion methods

ACKNOWLEDGEMENTS

The authors express their sincere appreciation to Ms Cynthia Banas for her expert advice on the development of the surgical procedures Thanks are also due to Dr Carlos Beyer for his valuable instruction concerning amino acid neurochemistry This work was supported by NIH grant NLS-2 5 Rol NS22948-03 (to B R K), as well as the Rutgers Busch Fund and CIBA-GEIGY (to F J)

REFERENCES

1 Davies, L P , Johnston, A R , Stephanson, A L Postnatal changes in the potassium-stimulated, calcium-dependent release of radioactive GABA and glycine from slices of rat central nervous tissue J Neurochem 25 387–392, 1975
2 Dudzinski, D S , Cutler, R W P Spinal subarachnoid perfusion in the rat glycine transport from spinal fluid J Neurochem 22 355–361, 1974
3 Falconer, D S Introduction to quantitative genetics New York Ronald Press, 1960 142–149
4 Franklin, G M , Dudzinski, D S , Cutler, R W P Amino acid transport into the cerebrospinal fluid of the rat J Neurochem 24 367–372, 1974
5 Haegele, K D , Schwartz, J -J , Schoun, J , Schmidt, A H , Schechter, P J 2-Pyrrolidinone in human cerebrospinal fluid a major constituent of total gamma-aminobutyric acid J Neurochem 49 1402–1406, 1987
6 Jones, B N , Gilligan, J P o-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of

polypeptide hydrolyzates and physiological fluids J Chromatogr 266 471–482, 1983
7 Kornhuber, M E , Kornhuber, J , Kornhuber, A W , Hartman, G M Positive correlation between contamination by blood and amino acid levels in cerebrospinal fluid of the rat Neurosci Lett 69 212–215, 1986
8 Levi, G Release of putative transmitter amino acids In Lajtha, A , ed Handbook of neurochemistry vol 6, 2nd ed New York Plenum Press, 1984 327–350
9 Martin, H , Kocher, L , Chery-Croze, S Chronic lumbar intrathecal catheterization in the rat with reduced-length spinal compression Physiol Behav 33 159–161, 1984
10 Masters, D B , Beyer, C , Jordan, F , Steinman, J L , Komisaruk, B R Evidence that vaginocervical stimulation releases amino acids into superfusates of the spinal cord Soc Neurosci Abstr 14 349, 1988
11 Oja, S S , Kontro, P Cation effects on taurine release from brain slices comparison to GABA J Neurosci Res 17 302–311, 1987
12 Potashner, S J Effects of tetrodotoxin, calcium and magnesium on the release of amino acids from slices of guinea-pig cerebral cortex J Neurochem 31 187–195, 1978
13 Sellstrom, A , Hamberger, A Potassium-stimulated gamma-aminobutyric acid release from neurons and glia Brain Res 119 189–198, 1977
14 Skilling, S R , Smullin, D H , Beitz, A J , Larson, A A Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation J Neurochem 51 127–132, 1988
15 Yaksh, T L , Rudy, T A Chronic catheterization of the spinal subarachnoid space Physiol Behav 17 1031–1036, 1976
16 Yaksh, T L , Tyce, G M Resting and K^+-evoked release of serotonin and norepinephrine in vivo from the cat and rat spinal cord Brain Res 192 133–146, 1980
17 Yaksh, T L The central pharmacology of primary afferents with emphasis on the disposition and role of primary afferent substance P In Yaksh, T L , ed Spinal afferent processing New York Plenum Press, 1986 165–195