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A manually-operated brain tissue slicer suitable for neurotransmitter release studies

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A simple, inexpensive device has been designed to prepare slices or prisms of brain tissue for metabolic and release experiments by means of a hand-operated method. It is particularly suitable for very rapid slicing of small, irregular pieces of brain tissue dissected from animals or specimens available at biopsy. This simple apparatus was produced as an alternative to the more widely used motor-driven tissue choppers. A comparison of the morphological and metabolic integrity of tissue produced by the new slicer and a conventional tissue chopper is presented. The hand tissue slicer has been used to measure the release of monoamine neurotransmitters and specific neuropeptides in vitro.

Introduction

Tissue slice techniques have been used for more than 50 years to study tissue metabolism. More recently, mammalian brain slices have been a particularly valuable tool for examining neurotransmitter release mechanisms because of the relative structural integrity, good respiratory performance and maintenance of neuronal excitability of brain slices (Yamamoto and McIlwain, 1966; Lynch and Schubert, 1980). Whilst careful slicing by hand is the usual preferred method (McIlwain, 1975) this technique is restricted by the difficulty of rapid preparation of small brain pieces of uniform dimensions from various brain regions with minimal trauma to the tissue. We have developed an instrument for rapid preparation of brain tissue slices which consists of a manually operated technique based on the 'egg slice' principle. The method avoids the trauma of cutting or chopping procedures characteristic of other methods (McIlwain, 1975; Duffy and Teyler, 1975; Hatton et al., 1980; Krumdieck et al., 1980) and can be used successfully by an inexperienced operator. In this paper, we describe the release of monoaminergic transmitters and the neuropeptide thyrotrophin-releasing hormone (TRH) from regional rat brain slices prepared by this new technique and compare the morphological appearance and

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metabolic performance of the slices with those of similar dimensions prepared using a McIlwain tissue chopper.

Materials and Methods

Description and operation of the slicer

The construction and appearance of the slicer is shown in Fig. 1. The body of the instrument is made from brass, which is then nickel- or zinc-plated. It consists of a base, to which a removable base plate is attached, and a hinged grid holder. The base plate contains a raised central area which is precision-grooved, using specially-designed manifold steel cutters, to the dimensions indicated. These dimensions were optimally determined for brain tissue from a series of prototypes of differing dimensions and the instrument produces slices or blocks of brain tissue of approximately 0.45 mm thickness in 2 dimensions. A grid of stainless-steel wire (0.125 mm diameter) is attached to the upper hinged portion. The grid is constructed from 2 lengths of wire, one for each of 2 dimensions consisting of 24 strands attached to each of 2 sets of 12 support pins (Fig. 1). The wires rest in grooves within the upper grid holder and are tightened by means of the wedged bridge clamps shown on the underside view of the upper grid holder (Fig. 1). In the closed position the wires of the grid rest within the grooves of the base plate as shown in Fig. 1 such that the lower set of wires are recessed below the level of the base and the upper set are only partly recessed.

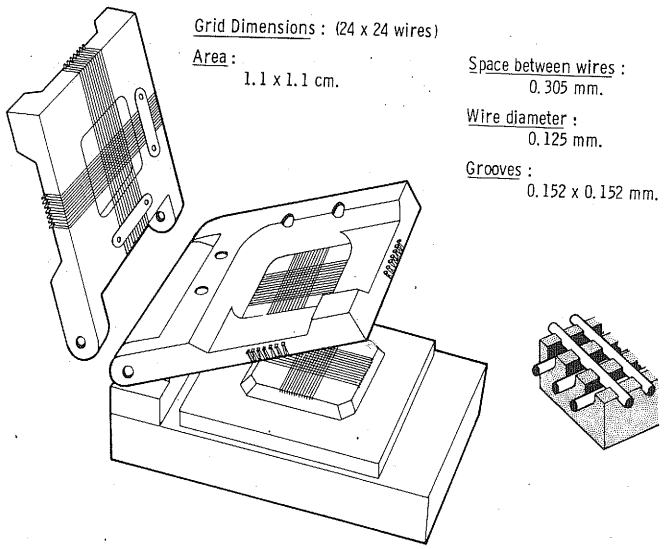


Fig. 1. The brain tissue slicer described in this paper, indicating the salient features of construction (see text for details).

The instrument is designed for very rapid slicing of small irregular pieces of brain tissue (10–100 mg). Following rapid dissection the tissue is placed on the centre of the base plate, bathed in a few drops of physiological saline or incubation medium, and sliced by firmly closing the wire grid which teases through the tissue to form the slices in one action. The tissue slices are carefully removed from above the closed grid using a fine spatula and immediately transferred to calcium-free incubation medium.

Pretreatment and incubation of slices for neurotransmitter / neuropeptide release studies

Slices were prepared from both halves of bilaterally divided tissue of various brain regions, weighed and placed in 2 ml calcium-free Krebs buffer, washed by gentle agitation and removed by centrifugation (5 min at 1000 g). The slices were then resuspended in 0.5 or 1 ml gassed (95% O_2; 5% CO_2) Krebs bicarbonate buffer, pH 7.4, containing glucose (10 mM), bacitracin (20 μM), and pargyline (50 μM) at tissue concentrations of 10–50 mg/0.5 ml or 50–100 mg/ml in small plastic capped vials. High potassium (KCl) was added in 10 μl buffer after 5 min incubation or 10 μl buffer added alone to the controls. The capped vials were then incubated for 20 min at 37°C in a shaking water bath, and at the end of the incubation period the slices were separated by centrifugation (5 min at 1000 g) and the supernatants stored in aliquots at −20°C for subsequent assay of amine or peptide.

Morphological examination of sliced and chopped rat brain tissue

Slices or prisms of rat brain tissue from striatum, septum and nucleus accumbens were prepared either with the new slicer or by using a McIlwain tissue chopper set to process slices of similar proportions. The tissue slices were either fixed immediately or incubated for 25 min in Krebs medium as described in above and then fixed.

Tissues were fixed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M PO_4 buffer pH 7.3 for 30 min, rinsed in cacodylate buffer and post-fixed in 2% OsO_4 in cacodylate buffer (pH 7.2) for 1 h. After dehydration the tissues were embedded in Araldite. Ultrathin sections were cut from comparable depths within the blocks of embedded tissue and contrasted sequentially with lead citrate and uranyl acetate and viewed in a Philips 3000 electron microscope.

Respiration of rat brain tissue slices

Oxygen uptake measurements of selected regional brain slices were determined with a temperature regulated Clarke oxygen electrode with a magnetic stirrer and attached pen recorder (Chappell, 1964). Slices were prepared from hypothalamus, striatum, nucleus accumbens and cerebral cortex using the new slicer as described above or alternatively slices of similar dimensions (0.45 × 0.45 mm) were prepared using a McIlwain tissue chopper (McIlwain, 1975). The slices were weighed and resuspended in fully aerated (95% O_2 : 5% CO_2) Krebs medium as described above at similar tissue concentrations and 2 ml of the resuspensions was added to the electrode chamber, following calibration with buffer and the use of crystals of sodium dithionite to remove the O_2 activity. Using the value of solubility of O_2 in the

medium at 37°C taken from tables, the oxygen uptake of gently stirred slice suspensions was determined as μmol O_2/g wet weight/h.

Measurement of the dopamine noradrenaline, 5-hydroxytryptamine and thyrotrophin-releasing hormone (TRH)

The monoamine neurotransmitters in the supernatant media were assayed by high-performance liquid chromatography (HPLC) with electrochemical detection using a modification of the methods described by Bennett et al. (1980) (Marsden et al., 1981). TRH was measured by a sensitive and specific radioimmunoassay also modified from the method of Bennett et al. (1980) (Bennett et al., 1982).

Results and Discussion

Morphological examination of sliced and chopped tissue

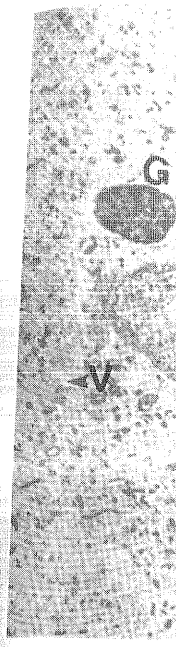
Slices of striatum and nucleus accumbens prepared using the new slicer and fixed before or after incubation displayed a high degree of integrity. The tissue did not show marked vacuolization or swelling of cells, although the neuropile was loosely packed and some membrane damage was evident (Fig. 2a). At a subcellular level the integrity of both neuronal and glia cell organelles such as mitochondria, Golgi complexes and ER appear well-preserved, as were nuclear membranes (Fig. 3a). Similarly, synaptic contacts can be clearly identified by the intact synaptic vesicles and membrane specializations (Fig. 3b). Prisms of septum and hypothalamus also displayed a similar degree of tissue preservation.

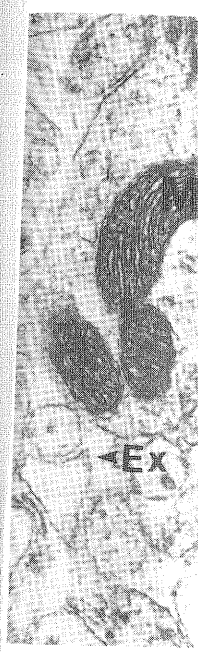
Slices from similar brain regions prepared using a McIlwain tissue chopper showed reduced tissue preservation and marked vacuolization after incubation. An example of this is seen in Fig. 2b. Similar results have been reported by Garthwaite et al. (1979).

Although general structural preservation of the tissue slices is good, it is inevitable that incubation and subsequent immersion fixation of the tissues produce a degree of structural change and increase in extracellular space not found in conventional perfused fixed material. This is particularly true of glia and glial processes which generally show a higher susceptability to damage than do neurones. However, the degree of structural integrity of the sliced tissue is clearly compatible with the physiological performance of the tissues.

Respiration of brain tissue slices

Table 1 shows the oxygen uptake of slices from selected brain regions determined with the oxygen electrode and expressed as μmoles O_2/g wet weight/h. Table 1A shows the oxygen uptake determinations of slices prepared with the new slicer from rat hypothalamus, striatum and nucleus accumbens. In all cases tested, this rate was increased 1.5- to 2.0-fold in the presence of potassium (56 mM). Table 1B shows the oxygen uptake determinations of slices prepared from rat cerebral cortex either with





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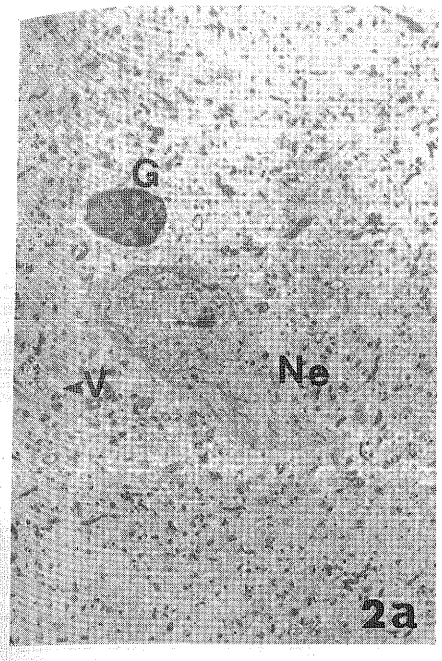
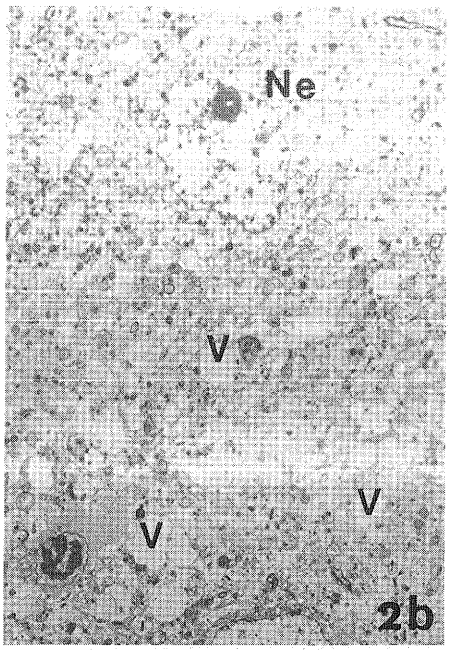
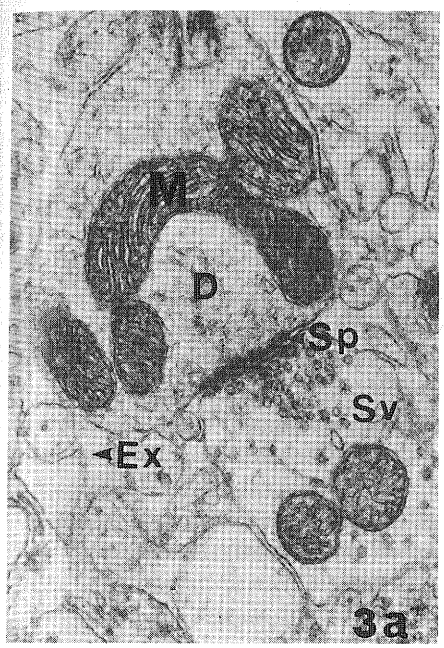






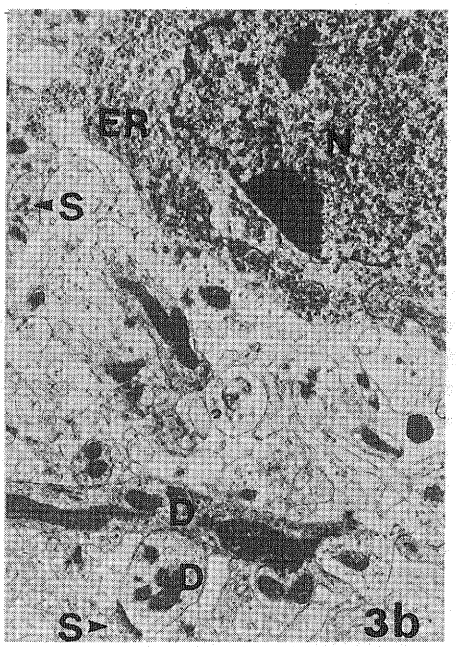


Fig. 2. a: sliced incubated striatum. Less vacuolization (V) of neuropile than in chopped material; good preservation of neurone (Ne) and glia (G). ×2046. b: chopped incubated striatum. Micrograph showing extensive vacuolization of neuropile (V); damaged neurone (Ne). ×2046.

Fig. 3. a: sliced incubated nucleus accumbens. Axo-dendrite synapse showing good preservation of synaptic specialization (Sp), mitochondria (M), dendrite (D), and synaptic vesicles (Sv). Note relative increase in extracellular space (Ex). ×26,000. b: sliced incubated nucleus accumbens. The nucleus (N), endoplasmic reticulum (ER), and cytoplasm show good preservation; also presynaptic terminal (S), dendrite (D). ×9500.

TABLE 1

OXYGEN UPTAKE OF SLICES FROM SELECTED BRAIN REGIONS

Oxygen uptake was determined with a Clarke-type oxygen electrode using weighed pieces of tissue (20–200 mg) sliced and resuspended in 2 ml of Krebs bicarbonate medium, pH 7.4, containing glucose (10 mM), bacitracin (20 μm) and pargyline (50 μM), fully aerated with 95% O_2:5% CO_2 at 37°C. Values represent μmol O_2/g.wet weight/h.

(A) Respiration of rat brain tissue slices

Brain area	Mean ± S.E.M. (n)	
Hypothalamus	61.2 ± 1.1 (5)	Addition of KCl (56 mM) caused a 1.5- to 2-fold increase
Striatum	75.1 ± 2.9 (5)	
Nucleus accumbens	73.9 ± 3.2 (3)	

(B) Respiration of cerebral cortex slices: comparison of oxygen uptake of tissue sliced or chopped

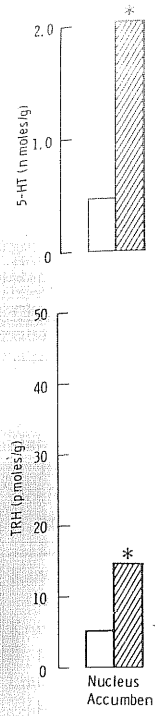
	Sliced (new slicer)	Chopped (McIlwain tissue chopper)
Control medium	62.9 ± 4.7 (4)	53.3 ± 4.1 (4)
Medium + KCl (56 mM)	119.4 ± 8.9 (4)	108.2 ± 8.5 (4)

the new slicer or by tissue chopper, together with the effect of incubation with potassium (56 mM). The values obtained indicate a higher respiratory performance of the sliced compared with the chopped tissue, although this is not statistically significant, and a 2-fold increase in the presence of potassium in each case.

The release of TRH and monoamine neurotransmitters from rat brain slices

The release of 5-HT and TRH from slices of nucleus accumbens, striatum, hypothalamus, septum, hippocampus and brainstem in response to elevated potassium is shown in Fig. 4. Potassium (56 mM)-induced 5-HT release occurred from all regions tested. The most marked responses were observed with hypothalamic and accumbens slices, two brain regions richly innervated by 5-HT-containing axons and nerve terminals (Steinbusch, 1981). Marked potassium-induced TRH release was seen only with slices of accumbens, hypothalamus and septum, which is consistent with the distribution of TRH-containing nerve terminals in rat brain as shown by immunohistochemical studies (Hökfelt et al., 1975) and tissue levels as shown by radioimmunoassay (Brownstein et al., 1974; Bennett et al., 1980). Potassium-stimulated TRH release occurred with striatal slices also, but not with hippocampal and brainstem slices.

The release of noradrenaline and dopamine from slices prepared from similar brain regions was measured (Fig. 5). In this case, the response to elevated potassium at 30 mM and 56 mM was determined, as was the effect of the absence of free calcium ions in the incubation medium. Significant potassium-induced release of noradrenaline (2- to 5-fold) occurred from slices of nucleus accumbens, hypothalamus, septum and hippocampus at 30 mM, 56 mM or at both concentrations as



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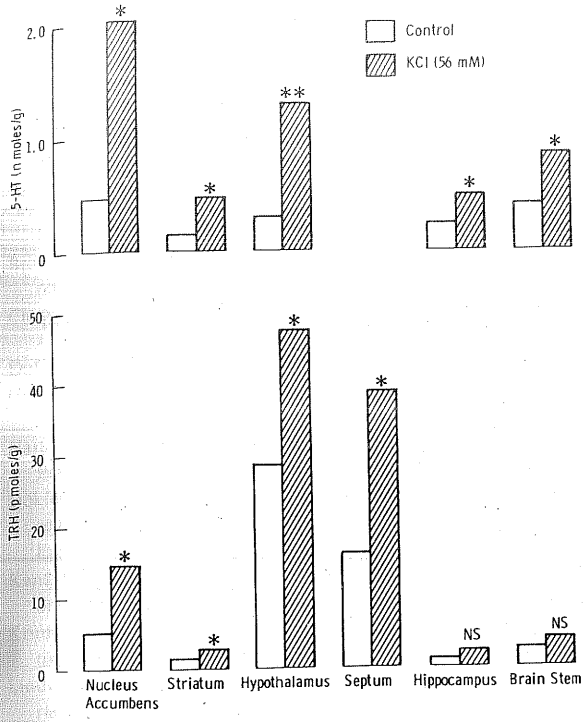

Fig. 4. Potassium-induced release of 5-hydroxytryptamine (5-HT) and thyrotrophin-releasing hormone (TRH) from slices of rat brain regions prepared with the novel slicer (see text for details). The open columns represent basal release while the shaded columns represent release in response to potassium (56 mM). Data were analyzed by Mann–Whitney U-test; * $P < 0.02$, ** $P < 0.002$ ($n = 5–9$).

indicated, and this release was inhibited in the absence of calcium ions. The release of noradrenaline from brainstem slices was not increased by potassium (56 mM) and no measurable basal or potassium-induced release of noradrenaline occurred with the striatal slices. Potassium (at 30 mM, 56 mM or both concentrations) markedly enhanced the release of dopamine (3- to 12-fold) from the slices of all 6 regions tested, particularly from accumbens, striatal, hypothalamic and hippocampal slices at the higher potassium concentration. Comparable with the release of noradrenaline, the induced release of dopamine was inhibited with the absence of calcium ions from the incubation medium. Again, the results of potassium-induced noradrenaline and dopamine release from the regional brain slices is consistent with the known distribution of the axon terminals of ascending catecholamine systems in rat brain (Lindvall and Björklund, 1974). Furthermore, the levels of dopamine release from the striatal slices are equivalent to those measured by electrochemical detection by Plotsky et al. (1977) using rat striatum slices (0.5–1.0 mm) prepared with a sharp razor blade. The brain regional release of catecholamines obtained with the novel slicer in this study also agree well with those of Kant and Meyerhoff (1978), who

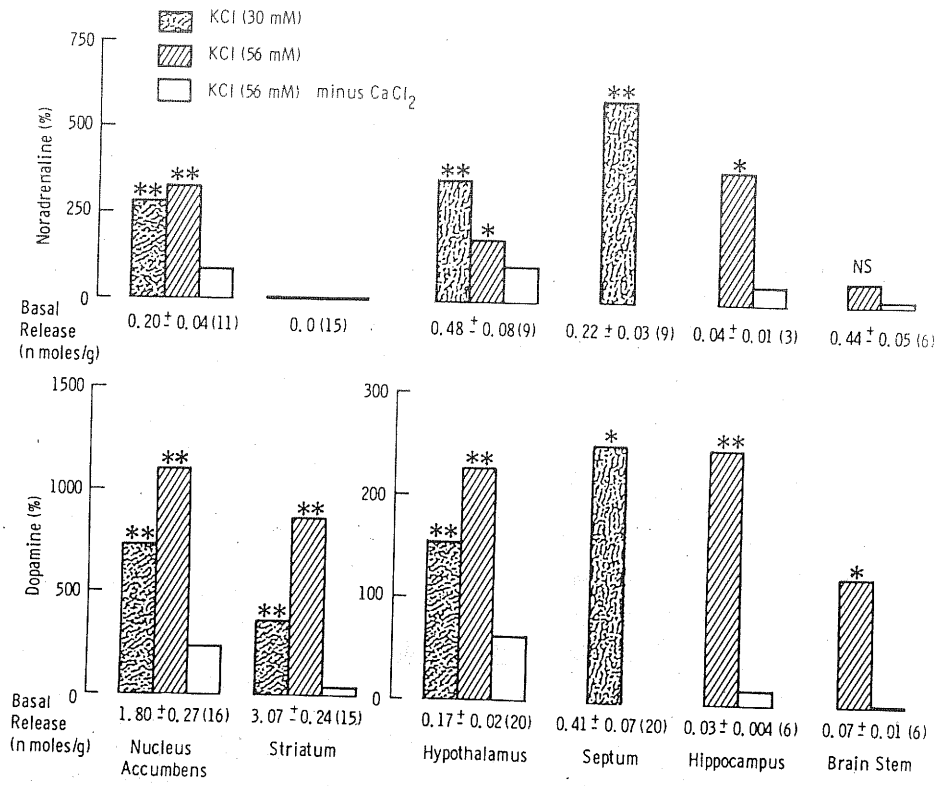

Fig. 5. Potassium-induced release of noradrenaline and dopamine from slices of rat brain regions prepared with the novel slicer (see text for details). The control basal release is represented as numerical values (means ± S.E.M.s (n)) and the blocks represent the percentage increase in release due to elevated potassium. The mottled shaded columns represent potassium (30 mM), the hatched shaded columns represent potassium (56 mM) and the open columns represent release in the presence of potassium (56 mM) with absence of calcium ions. Data were analyzed by Mann–Whitney U-test; * $P < 0.02$, ** $P < 0.002$.

measured endogenous amine release from rat brain slices using a radio enzymatic assay.

In conclusion, the evidence for structural integrity and metabolic performance of the sliced tissue together with the release studies confirm the efficacy of this new approach for the rapid preparation of regional brain slices.

Acknowledgements

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