Isethionate may not be an inert substitute for extracellular chloride in the central nervous system[1]

N. Bernardi, J. A. Assumpção[2], N. Davidson and C. G. Dacke

Department of Physiology, Marischal College, University of Aberdeen, Broad Street, Aberdeen AB9 1 AS (Scotland), 20 December 1976

Summary. Replacement of extracellular chloride with isethionate or methylsulphate causes an increased efflux of 1-[^{14}C]-GABA from the in vivo superfused rat cuneate nucleus. This raises the question of the suitability of these anions as inert substitutes for chloride in studies on the ionic dependency of membrane phenomena in the central nervous system.

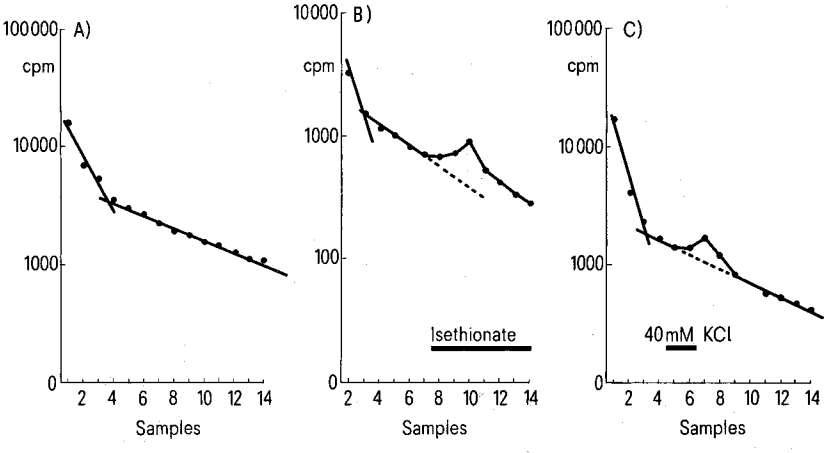
A number of anions such as sulphate[3], isethionate[4,5] and methylsulphate[6] have been used as relatively impermeant substitutes for extracellular chloride in a variety of electrophysiological investigations of the vertebrate central nervous system. Recently, however, we have obtained data which suggest that extracellular chloride replacement not only alters trans-membrane chloride gradients but also affects the release of at least one major central neurotransmitter, GABA (γ-amino-n-butyric acid). Such an effect could alter post-synaptic membrane electrical characteristics in a way not directly related to the change in chloride gradient.

Materials and methods. The preparation used was the cuneate nucleus of the chloralose-urethane anaesthetized rat, where pharmacological data suggest that GABA is a major inhibitory transmitter[7]. The method of superfusion of a restricted area of the exposed pial surface of the cuneate, labelling the endogenous GABA pool with 1-[^{14}C]-GABA and collection of subsequent superfusate fractions for estimation of isotope efflux has already been described[8]. After a 60 min period of incubation of the cuneate with a solution containing 2.0 μCi ml^{-1} of 1-[^{14}C]-GABA, the nucleus was superfused at 62.5 μl min^{-1} with an artificial cerebrospinal fluid (CSF) solution[9], a similar CSF but with an elevated (40 mM) potassium content, or with one in which the sodium chloride had been replaced with sodium isethionate. All solutions were buffered with Tris to pH 7.40 and osmolalities matched at 315 mosm/l. 5-min fractions of superfusate from the cuneate nucleus were collected serially in glass vials containing 6 ml Multisol II (Intertechnique Ltd.) and 0.1 ml distilled H_2O, and radioactivity estimated by liquid scintillation spectrometry. Counting efficiency was constant in these experiments, so that quench correction was unnecessary.

Results and discussion. Figure, A shows the spontaneous efflux of 1-[^{14}C]-GABA from the cuneate nucleus. Plotted semilogarithmically, the spontaneous efflux consists of 2 or more phases for which straight lines have been computed by the least squares method. The first, rapid phase, probably indicates extracellular wash-out, so the effect of changing the chloride content has been studied in the later, slower phase or phases.

Figure, B shows the effect of changing from normal to low-chloride CSF after collection of the 7th 5-min sample of cuneate superfusate. A straight line has been computed as before, but from the points corresponding to samples 4–7 only, the isethionate CSF reaching the cuneate from sample 8 onwards. It can be seen that between samples 8, 9, and 10 at least, the isotope content in the superfusate has sharply increased compared with the pattern seen in the spontaneous efflux (figure, A) indicating that low chloride superfusion has accelerated the efflux of GABA from the cuneate.

This is a consistent effect (9 experiments) but difficult to quantify because of the unpredictability of the precise shape of the GABA efflux pattern and variability in absolute size of the isethionate response from experiment to experiment.



Efflux of 1-[^{14}C]-GABA from the superfused cuneate nucleus. Rats were pretreated with amino-oxyacetic acid (AOAA, 2 mg/100 g b. wt) to reduced GABA catabolism. *A* spontaneous GABA efflux during continuous superfusion with normal CSF. Data are plotted semilogarithmically to show fast and slow efflux components. Lines of best fit are computed (see text). *B* GABA efflux during which normal CSF is replaced with low Cl^- (isethionate substituted) CSF at samples 8 onwards (horizontal bar). Lines of best fit are computed for samples 4–7 only and extrapolated (dotted line) to demonstrate increased GABA efflux under low Cl^- treatment. *C* GABA efflux during which normal CSF is replaced with one containing high (40 mM) K^+ during a 10 min period (horizontal bar). Lines of best fit are computed but missing out data from samples 6, 7 and 8. These show a similar increase in GABA efflux to that obtained with low Cl^- CSF.

A comparison of variance estimates[10] between 7 control and 9 isethionate experiments gives an F value = 15.2 with $p < 0.001$. We have therefore quantified the effect by comparing the percentage change in activity in sample 9 with that in sample 7 in both control and isethionate experiments, using the non-parametric Mann-Whitney test[10] (in the isethionate experiments sample 7 immediately preceded the beginning of isethionate CSF perfusion). The median increase in GABA efflux calculated in this way was 27% ($p < 0.01$). This can be compared with the effect of superfusion of the cuneate nucleus for a 10-min period with CSF with an elevated (40 mM) potassium content (figure,C) which produces a mean increase in GABA efflux of 30%[11]. The possibility that the isethionate-induced GABA efflux seen in the experiments reported here could have been due to some discrepancy in the potassium content of normal and isethionate CSF has been discounted. Potassium contents were measured by flame-emission spectrometry and found to be identical.

It is unlikely that the increase in GABA efflux is a specific pharmacological effect of the isethionate molecule. A similar effect has been observed in 2 preliminary experiments using methylsulphate instead of isethionate as a substitute for chloride. It is therefore possible that any impermeant substitute for chloride might have the same effect. This brings into question the usefulness of impermeant anions as replacement for extracellular chloride in electrophysiological studies in the central nervous system.

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Pancreatic polypeptide: A possible role in the regulation of food intake in the mouse. Hypothesis

Francine Malaisse-Lagae, J.-L. Carpentier, Y. C. Patel[1], W. J. Malaisse[2] and L. Orci[3]

Institute of Histology and Embryology, Geneva University School of Medicine, CH-1211 Genève 4 (Switzerland), 11 March 1977

Summary. Pancreatic polypeptide (PP) is a recently identified hormone produced by pancreatic endocrine cells. The islets of genetically obese mice (ob/ob, C57 BL/6J), which are suspected to lack a circulating satiety factor, contain relatively few of the PP-producing cells. Administration of bovine pancreatic polypeptide (bPP) reduces food intake and suppresses body weight gain in the hyperphagic obese mice. It is postulated that PP participates in the regulation of food intake in a manner as yet undefined.

Pancreatic polypeptide (PP) is a new hormone, first isolated from avian pancreas[4] and later identified in several mammalian species, including man[5,6]. In the pancreas, this hormone is stored in both insular and extrainsular cells, which can be distinguished from insulin-, glucagon-, and somatostatin-containing cells by immunohistochemical criteria[7-11]. In rats and mice, the PP-containing cells are particularly numerous in the islets located in the cephalic part of the pancreas, where they rank second in number after insulin-containing cells[12]. Although exogenous PP affects several parameters of hepatic and gastro-intestinal function[5,13,14], its physiological significance remains unknown. Because the plasma concentration of PP increases after feeding[15-18], we have begun an investigation on the possible role of PP in the regulation of food intake. The present part of the study was performed in hereditarily obese mice, in which hyperphagia is tentatively attributed to the lack of a circulating satiety factor[19].

Materials and methods. 18 obese (ob/ob) and 6 age-matched lean (+/+) mice of the C57 BL/6J strain were purchased

1 Medical Research Centre, Prince Henry's Hospital, Melbourne Australia.
2 Laboratory of Experimental Medicine, Brussels University, Brussels, Belgium.

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