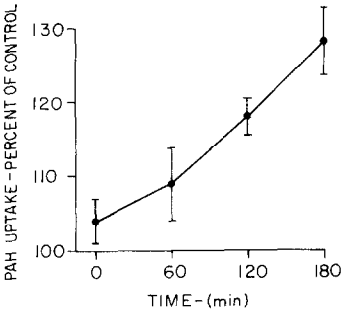

Fig. 1. Time course of the effect of PTH on PAH uptake. All tubules were incubated for 180 min and PTH was added at 0, 60, 120 or 180 min prior to the addition of PAH. Data represent mean ± S. E. M. of three experiments run in quadruplicate.

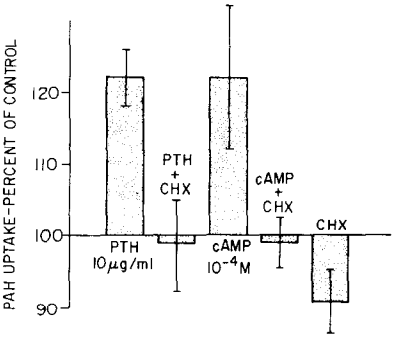

Fig. 2. Effect of cycloheximide (CHX) on the stimulation of PAH uptake by PTH and cAMP. Tubules were incubated as described for 130 min with either 10 μg/ml of PTH or 10^{-4} M cAMP. Some tubes also contained 1 mM cycloheximide. Note that cycloheximide has only partially reduced the stimulation of PAH uptake by PTH and cAMP because the PAH uptake in the tubules containing cycloheximide alone was reduced to 90 per cent of the control value. Data show mean ± S. E. M. of three experiments run in quadruplicate.

zide, another organic anion. Thus, it is likely that the effect shown in the present experiments results from stimulation of the renal transport system for organic anions. However, our experiments do not explain the mechanism of the stimulation of transport. While stimulation of PAH uptake is reduced by cycloheximide, there does not appear to be a lag phase during which stimulation does not occur, as seen in the stimulation of glucose and amino acid transport by DB-cAMP. The finding of such a lag phase has often been used to support a hypothesis that an effect is mediated via synthesis of new protein. Therefore, we cannot say at this point whether or not synthesis of transport protein might be involved. Furthermore, we cannot determine whether or not the effect of PTH is a direct one or is secondary to increased intracellular production of cAMP. However, the latter possibility is probably a reasonable working hypothesis until the exact mechanism of the stimulation of transport can be elucidated.

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Reduced incorporation of [^{3}H]leucine into cerebral proteins after long-term ethanol treatment

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Results concerning effects of long-term ethanol consumption on the incorporation of labelled amino acids into brain proteins of intact animals are scarce and conflicting [1,2]. In these studies ethanol solutions were given to the experimental groups instead of water. This procedure provides more 'empty' calories to the experimental animals than to the control animals. The subsequent reduced intake of protein or other essential nutrients by treated rats could thus be responsible for the apparent effect of ethanol on protein metabolism. Such effects could also vary among different experiments if the composition of the basic diet varied. The question was raised: whether ethanol intake could influence cerebral protein synthesis, independently of nutrition. In our experiments ehtanol was given to replace lipids isocalorically in a way which did not influence the daily intake of minerals, vitamins, protein or other essential nutrients.

Table 1. Effect of ethanol intake on incorporation of [^{3}H]leucine into cerebral proteins. The values are given ±SEM (standard error of the mean). NS = not statistically significant according to Wilcoxon's test, i.e. $\alpha > 0.05$

	Ethanol	Control	Significance
No. rats	6	6	—
Radioactivity in protein			
c.p.m./mg protein	79·6 ± 4·2	93·8 ± 2·7	$\alpha = 0.021$
c.p.m./g cerebral tissue	9·1 ± 0·5 × 10^3	10·7 ± 0·3 × 10^3	$\alpha = 0.021$
in % of control	85	100	—
TCA-soluble radioactivity			
c.p.m./g cerebral tissue	12·8 ± 0·8 × 10^3	12·6 ± 0·4 × 10^3	NS
cerebrum/plasma	0·77 ± 0·02	0·75 ± 0·01	NS

METHODS

Male albino Wistar rats (250–290 g initial body wt.) housed as described elsewhere [3] were fed a synthetic solid diet [3] *ad lib.* In addition the rats were 'pair'-fed (each 'pair' consisted of two experimental and two control rats) a liquid mixed diet containing either ethanol (16·7%, v/v) and sucrose (15%, w/v) (ethanol-group) or lipid (10%, w/v soybean oil) and sucrose (15%, w/v) (control-group). Pair-feeding was ensured by daily adjustments of the drinking volume offered. The mean daily consumption was 27 ± 1 ml fluid in both groups, and both ethanol as well as liquid lipids provided approximately 27 cal/rat/day in the respective groups. In this way the relative composition of the total diet consumed was calculated [4] to be (% of calories consumed): Ethanol: 30% (ethanol-group). Lipids: 35% (ethanol-group), 65% (control-group), Carbohydrates: 25% (both groups), Protein: 10% (both groups). The daily intake of vitamins, minerals, choline and methionine was equal in both groups and above the recommended values [5]. Rats of both groups grew well with no significant difference between the groups. The liquid diets were replaced by water 24 hr before sacrifice. 80 μCi of [4,5-^{3}H] L-leucine (TRK. 170, 46 Ci/m-mole, The Radiochemical Centre, Amersham) per kg rat was injected intraperitoneally one hour before decapitation at 10–11 a.m. The brains were frozen immediately in liquid nitrogen and stored at −20° until analysis. Blood was collected from the neck vessels, plama was separated and frozen until analysis. One hemisphere was homogenized in 20 vol. ice-cold 10% trichloroacetic acid (TCA). After centrifugation an aliquot of the supernatant was taken to determine TCA-soluble counts. The precipitate was washed, dissolved and counted as detailed elsewhere [3]. Protein concentration [6] was determined in the other hemisphere. One ml plasma was mixed with two ml 15% TCA and an aliquot was taken to determine TCA-soluble counts after protein precipitation. Unlabelled leucine was measured as described elsewhere [7].

RESULTS

Consumption of ethanol for 5 weeks caused a significant reduction of the specific radioactivity of cerebral proteins (Table 1, second line). The reduced incorporation was also present when referred to g cerebral tissue (Table 1, third line) since the protein concentration did not change significantly after ethanol treatment. Total brain weight was also unaffected by the consumption of ethanol. Ethanol treatment did not influence the amount of TCA-soluble counts present in cerebrum, nor the distribution of labelled leucine between plasma and cerebral tissue, measured one hour after the administration of isotope (Table 1, two lower lines). If this lack of difference was present throughout the labelling period, reduced leucine uptake could be excluded. The concentration of unlabelled leucine was not affected by previous ethanol treatment as the concentrations were (nmoles/g cerebral tissue): 108 ± 11 (ethanol) and 119 ± 28 (control), indicating no change of precursor specific radioactivity.

DISCUSSION

The present results could be due to reduced cerebral protein synthesis, but changes in protein degradation and leakage as well as changes of the metabolism of leucine could also explain our results. The lack of effect of ethanol on the TCA-soluble radioactivity does not rule out these latter possibilities, since [^{3}H]leucine is rapidly metabolized in brain tissue [8].

The conclusion is therefore that long-term consumption of ethanol alters protein or amino acid metabolism in the central nervous system. These alterations did not appear to be consequences of reduced intake of essential nutrients. The reduced intake of lipids by the ethanol-treated rats probably do not explain the reduced incorporation, since isocaloric substitution of lipids by sucrose did not affect [^{3}H]leucine incorporation into cerebral proteins (J. Mørland, unpublished observation).

Our observations are in agreement with the work of Noble and Tewari [2] on intact mice, showing reduced incorporation of labelled leucine into brain proteins after ethanol treatment for 1 to 3 months. Our results seem to be in disagreement with those of Jarlstedt [1], who found increased incorporation of labelled leucine into proteins of cerebral cortex 24 hr after cessation of a preceding period of ethanol consumption for 8 months. This could mean that the reduced incorporation found by Noble and Tewari [2] and in the present report was a transitory effect, if not dietary effects as discussed above, were responsible for the results or Jarlstedt [1].

Finally, due to methodological differences between the *in vivo* and the *in vitro* measurement of incorporation of labelled amino acids into protein, our results cannot easily be compared with those obtained *in vitro* [9].

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