inhibited by 10^{-7} M clorgyline, while the inhibition by 10^{-7} M deprenyl was about 30%. In addition, the inhibition patterns were almost single sigmoidal. These results indicate that 5-HT is deaminated mainly by type A MAO, demonstrating the presence of type A enzyme in mouse liver. When 1.0 mM 5-HT was used, the patterns were dramatically changed; a clear plateau appeared at 10^{-9}–10^{-7} M clorgyline. Consistent with the change in inhibition by clorgyline, the susceptibility to deprenyl increased at 1.0 mM of 5-HT. These data show that 5-HT is deaminated by both type of MAO in mouse mitochondria at high concentrations.

Figure 2 shows the inhibition of MAO in rabbit liver mitochondria, using different concentrations of 5-HT and tyramine as substrates. The results obtained with rabbit liver were generally similar to those for mouse liver; tyramine was deaminated mainly by type B MAO, while 5-HT at 20.0 µM mainly by type A and at 1.0 mM by both types. These data, therefore, demonstrate the presence of type A MAO in rabbit liver mitochondria.

In the literature[3,4], mouse and rabbit livers have been claimed to contain a single MAO form, type B, using tyramine and kynuramine as substrates. However, inhibition curves with non-specific substrates such as tyramine and kynuramine are not sensitive enough to detect 2 forms of MAO, if the activity of 1 form accounts for less than 10% of the total activity[14]. In fact, we also could not identify type A MAO in mouse and rabbit livers with only the aid of tyramine as substrate (figures 1 and 2). To solve this problem, we have used 5-HT as a type A substrate, and could successfully identify type A enzyme in both mouse and rabbit liver mitochondria.

In our previous papers[7,15,16] we have demonstrated that inhibition patterns are drastically changed, as regards different substrate concentrations, when β-phenylethylamine, phenylethanolamine and N-methylphenylethanolamine are used as substrates. This was also the case for 5-HT with mouse and rabbit liver mitochondria (figures 1 and 2). Thus, our present results lend further support to our warning[7,15] that substrate specificities of the 2 types should be evaluated over a wide substrate concentration range.

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Effect of prostaglandins and dibutyryl cyclic AMP on the morphology of cells in primary astroglial cultures and on metabolic enzymes of GABA and glutamate metabolism

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Summary. Prostaglandins (PGE_1) and dibutyryl cyclic AMP (dBc AMP) induce similar morphological changes in astrocytes obtained in primary cultures. PGE_1 and dBc AMP increased 2 enzymes of GABA and glutamate metabolism, GABA-T and AAT, but did not modify GDH and GLN-S. Prostaglandins probably affect the cAMP content of glial cells and act in the same way as dBc AMP on glial cell differentiation.

It is now generally accepted that glial cells play a role in synaptic activity regulation. Primary cultures of astrocytes are of major importance for the study of astrocytic development and function. Such cultures, obtained from newborn mice or rats, do not contain neuronal cells[1].

We have previously shown, using primary cultures of dissociated newborn mice brain hemispheres that astrocytes: a) contain a high level of GFA (2–3 µg/mg P), an astrocytic marker[2], and have a very low GAD activity (a neuronal marker); b) contain GABA-T, the GABA metabolizing enzyme, the activity of which increased during cell growth and was inducible by high extracellular GABA concentrations[3], c) show morphological changes when dBc AMP was added to the medium, which were associated with an increasing GABA-T activity[4].

Prostaglandins and particularly PGE_1 are known to affect neuroblastoma cells[5]. The sensitivity of glial cells to dibutyryl cyclic AMP (dBc AMP) and to PGE_1 are compared here, considering the morphological changes and some biochemical modifications concerning GABA and Glu metabolism induced in the presence of these 2 agents. The procedure for culturing glial cells has been described previously[3]. In brief, for the preparation of 10 cultures, cerebral hemispheres from 3 newborn Swiss mice were dissociated mechanically and passed through a sterile nylon sieve (80 µm pore size) into 10 ml of a modified Eagle's

minimum essential medium containing 20% fetal calf serum. The cultures were maintained at 37 °C in the presence of 5% CO_2 for several weeks with 3 medium changes per week; compounds tested were added to the culture medium.

The morphology of the cultures was checked under a Nikon phase contrast microscope. The basal level of GABA transaminase (GABA-T), the GABA metabolizing enzyme, aspartate aminotransferase (AAT), glutamate dehydrogenase (GDH) and glutamine synthetase (GLN-S) were measured during cell growth.

Immediately before each experiment, the medium was removed and cells were washed twice with a PBS solution.

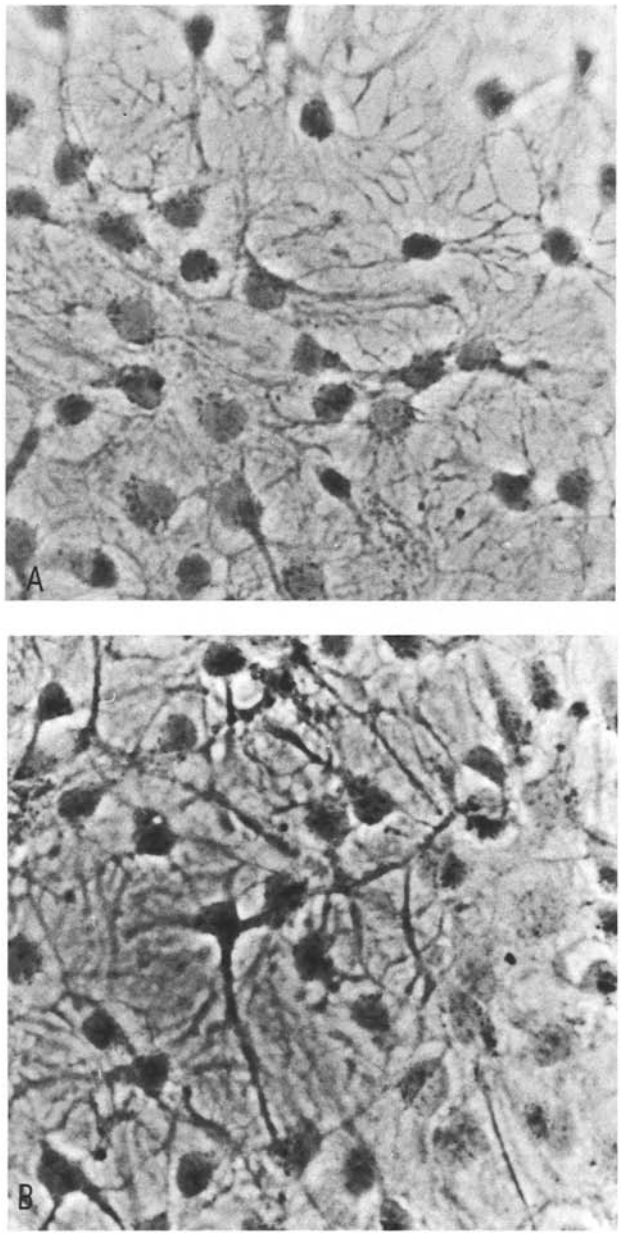

Fig. 1. Glial cells obtained by dissociated newborn mouse brain hemispheres cultured 15 days in vitro. Phase contrast, × 12. *A* 3 h after the application of 0.5 mM dBc AMP in the absence of calf serum (24 h before application). *B* 3 h after the application of 10 µg/ml PGE_1 in the absence of calf serum (24 h before application).

The layer of astrocytes was loosened with 0.6 ml of 0.05 M phosphate-acetate buffer (pH 6.8) containing PLP, DTT, EDTA at 10^{-4} M as protectors. The cells were broken by sonication 2 × 15 sec at 0 °C. Enzymes implicated in glutamate and GABA metabolism were investigated either during their growth period (for basal level) or during the confluent phase of culture (for PGE_1 and dBc AMP action). GABA-T, AAT, GDH were measured according to the method of Gonnard et al. using (1-^{14}C) ketoglutarate and GABA or aspartate as amino donor. (1-^{14}C) Glutamate formed was decarboxylated by adding *E. coli* decarboxylase and $^{14}CO_2$ was trapped and counted[6].

For GDH, the same assay was used, in the presence of NADH. GLN-S was measured according to Berl et al.[7]. Protein was measured by the technique of Lowry et al.[8]. All the enzymatic assays were performed at 37 °C, with 100 µl of cellular homogenate.

PGE_1 and also dBc AMP induced process formation were considered by Prasad to be an expression of morphological differentiation. PGE_1 at 10 µg/ml in the absence of foetal calf serum (FCS) stimulated the formation of 2 types of processes, the one grew in many directions and appeared on the protoplasmic type of astrocytes and the other, with 2 or 3 processes, characterized the fibrous astrocytes. These processes began to appear as early as 3 h after the addition of PGE_1 and were general after 24 h. No differences were observed at higher concentrations or during a longer time of contact. The same type of morphological changes were obtained in the presence of 0.5 mM of dBc AMP in the absence of foetal calf serum (figure 1). When cells were treated for a longer time with dBc AMP at 0.25 mM and in the presence of 10% FCS in place of 20%, changes appeared more slowly and were general after about 72 h treatment (not shown here). Remove of dBc AMP as well as of PGE_1 totally reversed the morphological changes.

GABA and glutamate metabolizing enzymes were investigated during a period of 40 days in culture or in the presence of PGE_1 or dBc AMP, after the confluent period (about 18 days).

The basal levels of the enzymes, measured for 5 weeks (figure 2), showed that the highest activity belongs to AAT

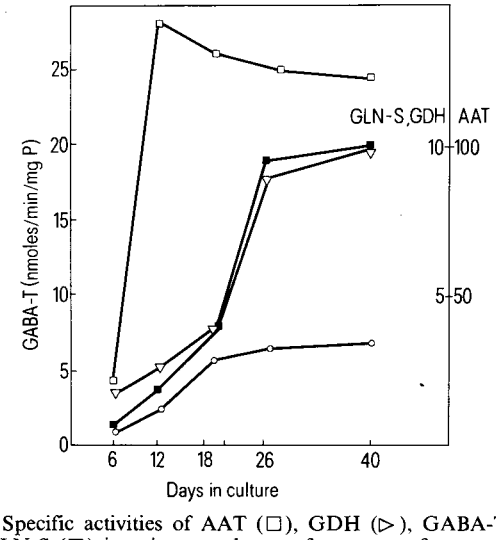

Fig. 2. Specific activities of AAT (□), GDH (▷), GABA-T (○) and GLN-S (■) in primary cultures of astrocytes from newborn mice as a function of the length of the culture period. Cultures were prepared as described by Bardakdjian et al.[4]. Results are averages of 5–7 individual experiments with SEM between 10 and 15% of the absolute value (for graphical reasons, the SEM are not shown).

which rapidly reached (1 week) the specific activity found in mature mouse brain; GABA-T increased more slowly and reached about 6 µmoles/min/mg protein which corresponds to 60% of the adult mouse brain activity. Glutamate dehydrogenase (GDH) as well as glutamine synthetase (GLN-S) increased regularly for 18 days and reached the value found in adult mouse brain during the 3rd week. The relatively high GLN-S found in these cells is in agreement with the fact that this enzyme is mainly localized in astrocytes. The GABA and glutamate metabolizing enzymes showed different growth patterns during the culture period. As they are all mitochondrial (or partially mitochondrial) enzymes, this observation seems to show the existence of different mitochondrial populations perhaps corresponding to the various types of astrocytes. If PGE_1 or dBc AMP are added to the growth medium, in the absence of FCS, for 3 h, the activities of GABA-T, the GABA metabolizing enzyme and AAT, the most concentrated Glu metabolizing enzyme, are increased. Increase in GABA-T and AAT activities are respectively $35\pm5\%$ and $50\pm3\%$ in the presence of dBc AMP at 0.5 mM and $30\pm3\%$ and $40\pm7\%$ in the presence of PGE_1 at 10 µg/ml. GDH and GLN-S did not vary, and longer action of the 2 agents (for 1 week) did not modify the activities of the 2 last enzymes.

Finally, prostaglandins are known to affect cAMP concentration in neurons[5]. In our experimental conditions, PGE_1 and dBc AMP have a similar effect on glial cells as well as on their morphology and metabolic activities. As in neurons, PGE_1 probably affects the cAMP concentration in glia. Modifications in neurons have been shown to be induced by a mechanism which includes new protein synthesis. Similar studies are now under investigation in our laboratory.

Abbreviations. AAT: aspartate aminotransferase (EC 2.6.1.1); GDH: glutamate dehydrogenase (EC 1.4.1.3.); GLN-S: glutamine synthetase (EC 6.3.1.2.); GABA-T: GABA-transaminase (EC 2.6.1.19.); GAD: 6L-glutamate decarboxylase (EC 4.1.1.15.); PLP: pyridoxal phosphate; GFA: glial fibrillary acidic protein; dBc AMP: dibutyryl cyclic AMP; FCS: foetal calf serum; PGE_1: prostaglandins I type.

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Variations des concentrations de cAMP intracellulaire de différents types de cellules après infection par le virus Sendaï ou le virus de la stomatite vésiculeuse[1,2]

Intracellular cyclic AMP in vesicular stomatitis or Sendaï virus infected cells

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Summary. In KB cells, MRC5 and adult skin fibroblasts infected by low doses of Sendaï virus, intracellular cyclic AMP levels rose and fell in the first hours following infection, then remained lower than basal level during at least 2 days in KB cells and adult skin fibroblasts. When compared to other viruses or cAMP inducers previously described, this effect appeared specific of Sendaï virus. Mechanisms and roles of cAMP variations are discussed. VSV-infected KB cells showed slightly decreased cAMP levels during the first hours following infection.

Alors que beaucoup de travaux ont été consacrés aux variations de cAMP dues à des hormones, catécholamines, prostaglandines, dans des cellules transformées ou non, très peu de résultats se rapportent aux variations de cAMP consécutives à une infection par un virus non transformant[4,5].

L'objet de ce travail est double: - caractériser le rôle éventuel des virus à RNA non transformants Sendaï et VSV dans l'altération du taux intracellulaire de cAMP; - comparer ces effets sur 3 types de cellules humaines: fibroblastes diploïdes embryonnaires MRC_5, fibroblastes de peau adulte, cellules KB hétéroploïdes tumorales en lignée.

Matériel et méthodes. Toutes les cellules sont cultivées dans des boîtes plastiques T 75. Les cellules KB sont cultivées en milieu de Earle à hydrolysat de caséine (Institut Pasteur) en tampon bicarbonaté (20 mmoles/l). Les cellules MRC_5 (BioMérieux) sont cultivées en milieu de Eagle (BME, Flow) en tampon bicarbonate (17 mmoles/l), additionné de pyruvate de sodium (1 mmole/l) et d'acides aminés non essentiels (0,5 mmoles/l) (Flow). Les fibroblastes de peau sont, soit cultivés comme les MRC_5 (figure 2), soit en milieu de Earle (MEM) en tampon Hépes (17 mmoles/l) (Flow), additionné pareillement de pyruvate et d'acides aminés non essentiels. Dans tous les cas, les milieux contiennent du sérum de veau 10% (Flow), de la pénicilline (100 U/ml) et de la streptomycine (100 µg/ml).

Le virus Sendaï (*Paramyxovirus influenzae* I, 10^{10} DI_{50}/ml) est inoculé dans le liquide allantoïque d'œufs de poule embryonnés de 10 jours, à raison de $10^4 DI_{50}$ chacun. Après incubation à 37 °C pendant 48 h, le liquide allantoïque est récolté: il sert à infecter les cultures cellulaires soit après centrifugation à $1000\times g/15$ min et élimination du culot (figure 1) soit après purification par adsorption sur hématies de poule selon la méthode de Homma[6] au terme de laquelle 50–75% du pouvoir infectieux initial a été conservé (figures 2 et 3). Les cellules en culture sont infectées un jour