

three diatom species we tested<sup>1</sup> occurred merely in response to alterations in the CO<sub>2</sub> concentration, or whether it was also influenced by the concomitant change in pH, cannot be resolved. However, analogous to our experimental design, under natural conditions any change in the CO<sub>2</sub> concentration of sea water inevitably goes hand in hand with a change in pH. Thus, a distinction between a CO<sub>2</sub>- and a pH-related response in growth rate is not critical for extrapolation of the experimental results to natural conditions. Naturally, we agree that from a physiological point of view this question is of particular interest.

The second point raised by Turpin, the need to distinguish between CO<sub>2</sub> limitation of photosynthetic rate and growth rate, is indeed relevant to the interpretation of phytoplankton stable carbon isotope compositions. Because we actually measured growth rate rather than short-term photosynthetic rate, however, our results provide neither a means nor a need to distinguish between

the two. In fact, the observed CO<sub>2</sub> limitation of growth rate<sup>1</sup> also implies CO<sub>2</sub> limitation of photosynthetic rate.

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ing that Glatt and Snyder<sup>11</sup> did not detect type 5 mRNA in the heart. Our solution hybridization data are in agreement with those of Ishikawa *et al.*<sup>5</sup>, which indicate that type 5 adenylyl cyclase mRNA is quite abundant in the heart.

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## Type 5 adenylyl cyclase distribution

**SIR** — Several groups have reported the identification of eight G<sub>s</sub>-sensitive adenylyl cyclases<sup>1–11</sup> which can be classified into five distinct families. One group reported that an adenylyl cyclase cloned from a striatal complementary DNA library is brain-specific and localized to the striatum<sup>11</sup>. We<sup>6</sup> and others<sup>5</sup> had cloned the same adenylyl cyclase and called it type 5. The tissue specificity of the expression of one type of a widespread G

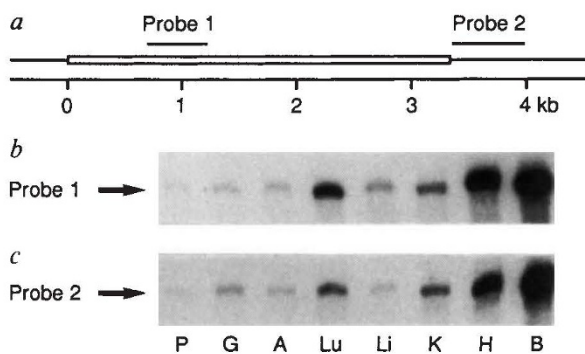
protein effector, such as adenylyl cyclase, is of potential importance.

Previous studies using PCR (polymerase chain reaction) had indicated a wide distribution for the type 5 and the related type 6 adenylyl cyclases<sup>6,9</sup>. It could be argued, however, that PCR is too sensitive and would detect very small and biologically inconsequential amounts of mRNA. Thus, we used solution hybridization to determine the presence of type 5 adenylyl cyclase mRNA in various tissues (see figure). Our probe 1 is a 500 base-pair fragment within the coding region, where the nucleotide sequence of the type 5 adenylyl cyclase is 82% similar to type 6. Probe 2 (a 620 base-pair fragment) encodes the 3' untranslated region<sup>12</sup> and is unique for the rat type 5 adenylyl cyclase (a in the figure). Total RNA from various rat tissues was extracted, and 10 µg from each tissue was used in the solution hybridization experiments. Both probes yield very similar patterns (b, c in the figure). We find that messenger RNA encoding type 5 adenylyl cyclase is most abundant in brain and heart, and is also found in pancreas, gut, adrenal gland, lung, liver and kidney. It is therefore surpris-

**GLATT AND SNYDER REPLY** — We identified a complementary DNA for an adenylyl cyclase selectively concentrated in the corpus striatum of the brain and thus probably responsible for the synaptic actions of dopamine<sup>1</sup>. The sequence of this DNA is essentially the same as the protein designated adenylyl cyclase type 5 and cloned independently from dog heart<sup>2</sup> and rat kidney<sup>3</sup>. Although our northern blot analysis did not reveal expression in peripheral tissues or other brain regions, *in situ* hybridization showed the striatal adenylyl cyclase in the heart and kidney, associated with blood vessels<sup>1</sup>. We have now confirmed these localizations, finding the striatal adenylyl cyclase in the heart concentrated in the atria, aorta and pulmonary artery (unpublished data).

In the pituitary gland we observe the enzyme localized particularly to the anterior lobe. In the kidney, messenger RNA encoding the enzyme is higher in the medulla than the cortex, and associated with tubules. In the eye, the striatal adenylyl cyclase is localized to the retina, both results agreeing with our initial conclusion that the striatal adenylyl cyclase is associated with dopamine's actions<sup>1</sup>. Thus, dopamine neurons and receptors are localized in the retina of the eye<sup>4</sup>, in the atria and great vessels of the heart<sup>5</sup>, in the anterior pituitary gland<sup>6</sup> and in the kidney<sup>7</sup>.

Pleroni *et al.* have conducted solution hybridization techniques and detect RNA



Detection by solution hybridization of mRNA encoding type 5 adenylyl cyclase in various tissues of the rat. a, Location of probes along the cDNA for type 5 adenylyl cyclase. b, Solution hybridization analysis of total RNA from pancreas (P), gut (G), adrenal gland (A), lung (Lu), liver (Li), kidney (K), heart (H) and brain (B) using probe 1. Type 5 enzyme mRNA is detected in all tissues. c, Solution hybridization analysis using probe 2. RNA was isolated by the guanidinium thiocyanate method. <sup>32</sup>P-labelled cRNA probes were prepared, and their sizes were verified by electrophoresis on an acrylamide gel. Samples of total RNA (10 µg) from each tissue were analysed for the presence of type 5 adenylyl cyclase mRNA by solution hybridization/RNase protection assay. After digestion, the samples were resolved on an acrylamide gel. Autoradiographs of the gel are shown.