(*12*) showed that 1,3-dimethyluric acid and 3-methylxanthines were produced in addition to caffeine (Fig. 2). However, the amount of caffeine produced was five times greater than the oxidative metabolites, suggesting that the predominant pathway of theophylline metabolism in the human fetus is methylation.

The presence of active methylation reactions (*11, 13, 14*) and the low activity of the hepatic microsomal oxidative enzymes in the human fetus and newborn infant (*15*) helps to explain the production of caffeine in neonates that are given theophylline (*4*). Our report provides evidence that caffeine is not only produced by the fetal liver but could be the major metabolite of theophylline.

It is intriguing to relate these results to the exceedingly slow elimination of caffeine in the newborn infant (mean half-life in plasma, 102 hours compared to 6 hours in nonsmoking adults) (*16, 17*) and to the faster elimination of theophylline in the neonate (mean half-life in plasma, 30 hours; adult value, 6 hours) (*1, 18*). The continued methylation of the dimethylxanthines to caffeine with negligible demethylation or C_8 oxidation of caffeine (*12, 19*) could result in caffeine's accumulation in plasma and slower elimination in the premature infant (*16*).

It is possible that theophylline acquired transplacentally, as when the mother drinks tea, may contribute to the fetal (and neonatal) caffeine load (*5, 20*). Direct administration of theophylline to newborn infants with apnea results in concentrations of caffeine in plasma that can elicit significant pharmacotherapeutic activity (*1*). Thus, the pharmacologic effects in infants treated with theophylline may in part be contributed by caffeine.

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References and Notes

1. J. V. Aranda and T. Turmen, *Clin. Perinat.* **6**, 87 (1979).
2. H. H. Cornish and A. A. Christman, *J. Biol. Chem.* **288**, 315 (1957).
3. J. W. Jenne, H. T. Nagasawa, R. D. Thompson, *Clin. Pharmacol. Ther.* **19**, 375 (1976).
4. C. Bory, P. Baltassat, M. Porthault, M. Bethenod, A. Frederich, J. V. Aranda, *Lancet* **1978-I**, 1204 (1978).
5. ——, *J. Pediatr.* **94**, 988 (1979).
6. S. Sved, R. D. Hossie, R. McGilvray, *Res. Commun. Chem. Pathol. Pharmacol.* **13**, 185 (1976).
7. L. R. Haber, V. Vaupshas, B. B. Vitullo, T. E. Seemayer, R. C. deBelle, *Gastroenterology* **74**, 1214 (1978).
8. C. Mitoma, L. Lombrozo, S. C. LeValley, F. Dehn, *Arch. Biochem. Biophys.* **134**, 434 (1969).
9. S. M. Lohman and R. P. Miech, *J. Pharmacol. Exp. Ther.* **196**, 213 (1976).
10. J. V. Aranda and T. A. Louridas, unpublished data.
11. G. E. Gaull, W. Von Berg, N. C. R. Raiha, J. A. Sturman, *Pediatr. Res.* **7**, 527 (1973).
12. A. Aldridge, J. V. Aranda, A. H. Neims, *Clin. Pharmacol. Ther.* **25**, 447 (1979).
13. L. Gluck, M. U. Kulovich, A. I. Eidelman, L. Cordero, A. F. Khazin, *Pediatr. Res.* **6**, 81 (1972).
14. L. Stern, M. H. Lees, J. Leduc, *Pediatrics* **36**, 367 (1965).
15. J. V. Aranda, S. M. MacLeod, K. W. Renton, N. R. Eade, *J. Pediatr.* **85**, 534 (1974).
16. J. V. Aranda *et al.*, *ibid.* **94**, 663 (1979).
17. W. D. Parsons and A. H. Neims, *Clin. Pharmacol. Ther.* **24**, 40 (1978).
18. J. V. Aranda, D. S. Sitar, W. Parsons, P. M. Loughnan, A. H. Neims, *N. Engl. J. Med.* **295**, 413 (1976).
19. M. G. Horning, C. M. Butler, J. Nowlin, R. Hill, *Life Sci.* **16**, 651 (1975).
20. L. F. Soyka and A. L. Neese, *Clin. Pharmacol. Ther.* **23**, 130 (1978).

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Choline Excites Cortical Neurons

Abstract. *In cats under halothane or methoxyflurane, iontophoretic applications of choline are only eight times weaker than applications of acetylcholine in evoking firing of neurons in the sensorimotor region of the cerebral cortex. The action of choline is suppressed by atropine but not by two agents that block choline uptake (hemicholinium-3 and triethylcholine), and is not potentiated by an anticholinesterase (physostigmine). Choline therefore appears to excite cortical neurons by a direct action, which may be a significant component of its beneficial therapeutic effects.*

As a precursor of acetylcholine (ACh), choline is an essential factor in cholinergic transmission (*1*). According to several recent reports (*2*), some patients suffering from tardive dyskinesia and possibly other disorders are significantly improved by ingestion of relatively large doses of choline or lecithin (from which choline is freed after absorption). These patients thus appear to suffer from a deficiency in cholinergic transmission in the brain, and the beneficial action of choline is thought to be mediated by an increase in neuronal ACh available for release from cholinergic nerve endings (*3, 4*). Another possible mechanism must be kept in mind. Choline is itself an ACh agonist (*5*), and therefore large doses of choline, which are known to raise greatly choline levels in the brain (*3, 4*), might have a significant direct action on cerebral neurons. In our first systematic investigation of cortical cholinoceptive neurons (*6-8*), choline was reported to be a weak excitant of ACh-sensitive cells. In view of the present great interest in choline and its clinical use (*9*), it seemed important to reinvestigate this topic in more detail. Judging by the relative intensities of equipotent iontophoretic currents, choline appears to be quite a strong excitant of ACh-excitable neurons, being only some eight times weaker than ACh itself.

Multibarrel glass micropipettes (*10*) were inserted into the pericruciate cortex of cats under light gaseous anesthesia (*11*). Under these conditions, neurons that are situated in the deeper cortical layers and discharge spontaneously in a characteristic manner (*6*) are particularly sensitive to ACh; as shown by the record of firing frequency of such a neuron in Fig. 1, these cells are often strongly excited by iontophoretic applications of 14 nA or less [probably equivalent to release of < 50 fmole/sec (*12*)]. When choline was released from another barrel of the micropipette, a response was obtained that was comparable in both intensity and time course (note especially the prolonged aftereffect), but a greater application of choline was almost always necessary: in Fig. 1, equipotent iontophoretic currents differed by a factor of 10 (it is also evident from Fig. 1 that the excitant action of choline compares quite favorably with that of glutamate released by an identical but briefer iontophoretic current). The mean ratio of

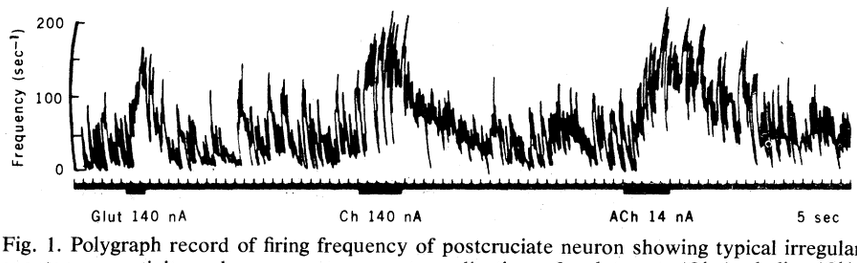

Fig. 1. Polygraph record of firing frequency of postcruciate neuron showing typical irregular spontaneous activity and responses to separate applications of L-glutamate (*Glut*), choline (*Ch*), and acetylcholine (*Ach*). Intensity and duration of microiontophoretic applications are indicated.

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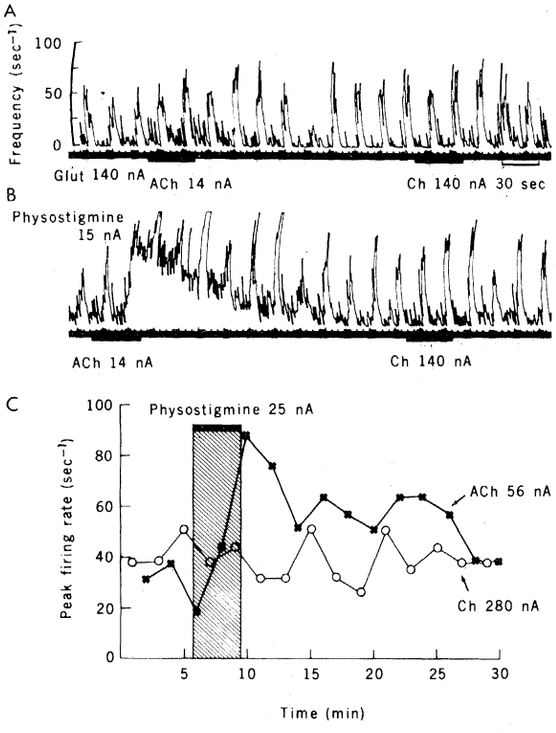

Fig. 2. Physostigmine selectively potentiates responses to ACh but not choline. (A and B) Postcruciate unit showing only minimal spontaneous activity but excited at regular 20-second intervals by brief (5-second) applications of glutamate. In (A) additional applications of ACh and choline evoked mainly a small increase in background activity. Record (B) was obtained 6 minutes after starting a steady and continuing release of physostigmine, which led to a progressive increase in responses evoked by ACh but did not enhance those produced by choline. (C) Data from another cell again indicate selective enhancement of ACh-evoked responses; in this series there were no applications of glutamate.

equipotent currents (*12*) of choline and ACh, applied for a similar period to 34 different neurons (in ten cats) was 8.2 (standard deviation, 5.12). [A further point of interest is that choline depressed the firing of a smaller population of cells that were inhibited by ACh (*13*).]

The excitatory effect of choline, like that of ACh (*6-8*), was readily and completely blocked by atropine, administered either directly by iontophoresis or intravenously (1 mg/kg). Another characteristic was a pronounced sensitivity to the depth of anesthesia, a two- to five-fold increase in the concentration of halothane or methoxyflurane leading to a sharp reduction in responsiveness to both ACh (*6, 14*) and choline.

These observations strongly indicated activation of the same postsynaptic muscarine receptors, but they did not exclude a possible indirect mechanism of action of choline, particularly through its uptake and conversion to ACh, thus making more ACh available for release—as emphasized especially by Wurtman and co-workers (*3*). [An action on presynaptic muscarine receptors was unlikely since activation of these tends to reduce ACh release (*15*).] If choline acted predominantly by increasing ACh release, rather than directly on postsynaptic receptors, then the inhibition of cholinesterase activity in the cortex should potentiate the action of both ACh and choline. In several experiments, physostigmine was released from the micropipette in conjunction with relatively small applications of choline and ACh. The results were entirely unambiguous. On every occasion physostigmine greatly potentiated the action of ACh but not that of choline. Two examples of such tests (on two different cells) are illustrated in Fig. 2. In Fig. 2, A and B, the neuron was excited throughout by regular brief applications of glutamate, and initially the applications of ACh and choline were just above threshold, evoking only a minor facilitation in glutamate-evoked responses and some increase in background firing (Fig. 2A). After several minutes of physostigmine ejection, the response to ACh was enormously increased, while that to choline remained essentially unchanged (Fig. 2B). The graph in Fig. 2C shows a comparable selective enhancement of ACh-evoked firing of another cell.

In a second type of experiment, we applied hemicholinium-3 (HC-3), a well-known blocker of choline uptake that should prevent any enhancement of ACh stores available for release (*1*). [In some experiments we used triethylcholine instead, for the same purpose (*16*).] There was no evidence of a specific depression of the excitatory effects of choline (in some instances, triethylcholine had an atropine-like action on both ACh and choline responses). On the contrary, these agents (HC-3 particularly) had a slow, ACh-like excitatory action, as previously reported (*17*), or potentiated the actions of choline and ACh.

We therefore conclude that choline has a marked excitatory action in the cerebral cortex, which is probably exerted through muscarine receptors. Although the iontophoretic technique does not provide a reliable estimate of the effective concentrations attained in the tissue, these observations indicate that at least some central ACh-sensitive neurons are sensitive to choline. It is therefore possible that large systemic doses of choline may directly activate many postsynaptic muscarine receptors in the central nervous system. This could explain why choline appears to be effective therapeutically even though there is no evidence that it alters the turnover of ACh in the brain (*18*).

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References and Notes

1. R. Birks and F. C. MacIntosh, *Can. J. Biochem. Physiol.* **39**, 787 (1961); F. C. MacIntosh and B. Collier, *Handb. Exp. Pharmacol.* **42**, 99 (1976).
2. K. L. Davis, L. E. Hollister, J. D. Barchas, P. A. Berger, *Life Sci.* **19**, 1507 (1976); J. H. Growdon, M. J. Hirsch, R. J. Wurtman, W. Wiener, *N. Engl. J. Med.* **297**, 524 (1977); R. J. Wurtman, M. J. Hirsch, J. H. Growdon, *Lancet* **1977-II**, 68 (1977).
3. E. L. Cohen and R. J. Wurtman, *Life Sci.* **16**, 1095 (1975); I. H. Ulus and R. J. Wurtman, *Science* **194**, 1060 (1976).
4. D. R. Haubrich, P. F. L. Wang, D. E. Clody, P. W. Wedeking, *Life Sci.* **17**, 975 (1975).
5. H. H. Dale, *J. Pharmacol. Exp. Ther.* **6**, 147 (1914); P. Trendelenburg, *Handb. Exp. Pharmakol.* **1**, 564 (1923); H. C. Chang and J. H. Gaddum, *J. Physiol. (London)* **79**, 255 (1933).
6. K. Krnjević and J. W. Phillis, *J. Physiol. (London)* **166**, 296 (1963).
7. ———, *ibid.*, p. 328.
8. Evidence that the central ACh-sensitive cells receive cholinergic innervation has been summarized by K. Krnjević [*Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 113 (1969); *Physiol. Rev.* **54**, 418 (1974)]. Many other central neurons give comparable (although not necessarily identical) ACh responses; therefore, even if cortical cells are not involved in tardive dyskinesia, the present observations are likely to be relevant for other central cholinergic systems that may be affected in pathological states.
9. G. B. Kolata, *Science* **203**, 36 (1979).
10. The electrodes were five- or seven-barrel glass micropipettes with tips of 5 to 10 μm. They were usually filled with 2*M* NaCl (for recording extracellular unit spikes) and 1*M* solutions of acetylcholine chloride (British Drug Houses), choline chloride (Sigma), and sodium L-glutamate (Sigma). Some electrodes also contained 0.1*M* physostigmine sulfate (Sigma), 0.1*M* atropine sulfate (Sigma), 0.05*M* hemicholinium-3 bromide (provided by B. Collier), and 0.05*M* triethylcholine iodine (Aldrich). A barrel containing 1*M* NaCl was routinely used for control tests for current artifacts.
11. Anesthesia was induced with 2 to 4 percent halothane in a 1:2 mixture of O_2 and N_2O and maintained with either halothane or methoxyflurane. After the dissection was completed, the dose of anesthetic was reduced to the minimum needed to keep the cat unperturbed (usually 0.5 percent halothane or 0.2 percent methoxyflurane). The animals were not paralyzed and breathed spontaneously. The femoral arterial pressure was monitored continuously.
12. Ratios were calculated from the currents needed to obtain submaximal responses of similar amplitude. A systematic series of tests over a wide range of response amplitudes could not be done regularly because of the need to space tests at long intervals—due to the long duration of responses—and the variability of background activity and responsiveness in these lightly anesthetized animals. Whenever sufficient data were available to draw dose-response curves,

these indicated comparable, approximately parallel curves for ACh and choline. Although we have not determined the transport number of choline during microiontophoresis, it is not likely to differ greatly from that of ACh [probably 0.1 to 0.4, according to K. Krnjević, J. F. Mitchell, J. C. Szerb, *J. Physiol. (London)* **165**, 421 (1963), V. E. Dionne, *Biophys. J.* **16**, 705 (1976)]. The ratios of equipotent currents are therefore likely to approximate the ratios of the equipotent amounts of choline and ACh released from the micropipettes.
13. M. Randić, R. Siminoff, D. W. Straughan, *Exp. Neurol.* **9**, 236 (1964); J. W. Phillis and D. H. York, *Brain Res.* **10**, 297 (1968).
14. R. F. H. Catchlove, K. Krnjević, H. Maretić, *Can. J. Physiol. Pharmacol.* **50**, 1111 (1972).
15. There is compelling evidence for presynaptic muscarine receptors, which tend to reduce ACh release in the cortex [R. L. Polak, in *Drugs and Cholinergic Mechanisms in the CNS*, E. Heilbronn and A. Winter, Eds. (Research Institute of National Defence, Stockholm, 1970), p. 323; J. C. Szerb, *Adv. Behav. Biol.* **24**, 49 (1978)].
16. W. C. Bowman and M. J. Rand, *Br. J. Pharmacol.* **17**, 176 (1961); W. C. Bowman and I. G. Marshall, in *International Encyclopedia of Pharmacology and Therapeutics*, J. Cheymol, Ed. (Pergamon, Oxford, 1972), sect. 14, vol. 1, p. 357.
17. K. Krnjević, R. Pumain, L. Renaud, *J. Physiol. (London)* **215**, 223 (1971). These atropine-sensitive excitatory effects of HC-3 may be explained by either a direct activation of muscarine receptors by HC-3 or a rise in concentration of extracellular choline secondary to block of choline uptake.
18. S.-A. Eckernäs, L. Sahlstrom, S.-M. Aquilonius, *Acta Physiol. Scand.* **101**, 404 (1977).
19. W.R. is a fellow of the Deutsche Forschungsgemeinschaft. We are grateful to the Canadian Medical Research Council for financial support and to F. C. MacIntosh and B. Collier for helpful discussions.

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Endocrine Pancreas: Three-Dimensional Reconstruction Shows Two Types of Islets of Langerhans

Abstract. *Three-dimensional reconstructions of islets of Langerhans, based on immunofluorescent staining of successive serial sections with antiserums to insulin, glucagon, somatostatin, and pancreatic polypeptide reveal a marked difference in the number of cells containing glucagon and pancreatic polypeptide depending on the anatomical location of the islet in the pancreas. The two types of islets are situated in regions of exocrine tissue that are drained by different excretory ducts. This demonstration contradicts the assumption that all islets in the pancreas are similar in their endocrine cell content.*

Recent textbooks in histology (*1*) describe the endocrine pancreas as a collection of minute secretory masses, called islets of Langerhans, that are formed of insulin-containing cells (most abundant), glucagon-containing cells, and somatostatin-containing cells (least abundant). Islets of Langerhans are said to be dispersed randomly in the exocrine tissue and to be more numerous in the body and tail of the gland than in the head. This report demonstrates that this view is largely incomplete.

In the rat, the pancreas is an irregularly shaped, elongated organ extending between the duodenum and the spleen. It is drained by at least two main excretory ducts (*2*) which open into the biliary duct at various levels. Injection of India ink (*3*) into the main proximal (or dorsal) duct, which drains into the biliary duct closest to the liver, results in the blackening of at least two thirds of the gland including the tail, body, and upper part of the head (Fig. 1A). Injecting the main distal (or ventral) exocrine duct, which opens into the hepatic duct closest to the duodenum, stains the remaining third of the pancreas (the lower part of the head) (Fig. 1C). Figure 1B illustrates diagrammatically the paths of these ducts.

A rat pancreas was fixed by perfusion with Bouin's fluid, and a piece (1 by 0.5 by 0.3 cm) was removed from the region drained by the dorsal duct, dehydrated, and embedded in paraffin. Three hundred serial sections, each 3 μm thick, were cut from the block; one of every 25 was stained with hemalum-eosin to ascertain the presence of well-preserved islets of Langerhans (the islets in this region are referred to as dorsal islets). The sampled areas yielded several islets completely or incompletely cut by the serial sectioning. From these islets, one was selected for reconstruction on the basis of completeness and quality. Successive groups of four consecutive sections in this series (77 sections were needed to include the entire selected islet) were stained by the immunofluorescence technique of Coons *et al.* (*4*), with specific antiserums to insulin, glucagon, somatostatin, and pancreatic polypeptide (*5*) being used. Color photographs from each immunofluorescent stained section were copied to scale onto Lucite sheets (Fig. 2, A to D) (*6*). The numbers of each endocrine cell type on each sheet were counted. Of 3126 cells counted, 2063 (66 percent) contained insulin, 874 (28 percent) contained glucagon, 123 (4 percent) contained somatostatin, and 66 (2 percent) contained pancreatic polypeptide. Nine islets stained in the same sections showed qualitatively the same distribution.

A similar reconstruction procedure was applied to one of the 16 ventral islets present in serial sections of a block taken from the lower part of the pancreatic head (Fig. 2, E to H), which is drained by the main ventral exocrine duct. In 68 serial sections evaluated, 4136 cells were counted, of which 3061 (74 percent) contained insulin, 818 (20 percent) contained pancreatic polypeptide, 190 (4 percent) contained somatostatin, and 67 (< 2 percent) contained glucagon (*7*).

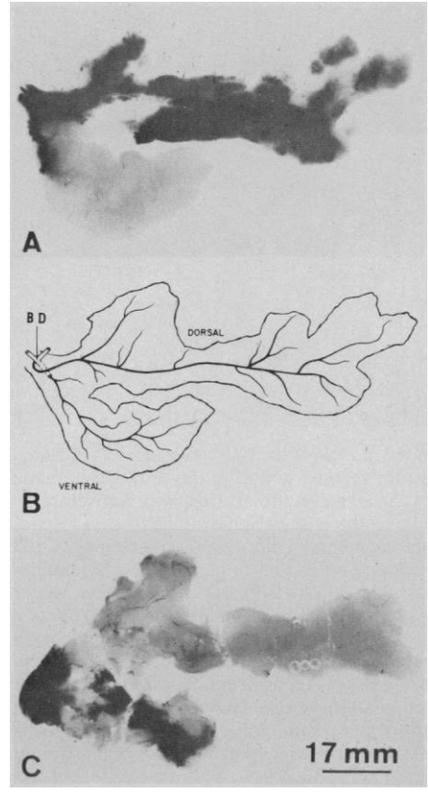

Fig. 1. (A) Thick longitudinal section in paraffin of an entire rat pancreas following injection of India ink into the main dorsal exocrine duct. Approximately two-thirds of the gland is stained, including the tail (right), body, and superior part of the head (left). The remaining third, the inferior part of the head, is not stained. The stained region yielded islets of Langerhans rich in glucagon-containing cells and poor in pancreatic polypeptide-containing cells (see Fig. 2, A to D) (×1.3). (B) Diagram of a pancreas showing pancreatic ducts injected with a mixture of India ink and latex (*3*). The main dorsal duct draining the part of the pancreas stained in (A) opens into the biliary tract at the point where the two hepatic ducts merge to form a single biliary duct (*BD*). The main ventral duct draining the lower part of the pancreatic head [see (C)] opens into the biliary duct below the entry of the main dorsal duct and close to the duodenum (not shown). (C) Thick longitudinal section in paraffin of an entire rat pancreas following injection of India ink into the main ventral exocrine duct. Only the lower part of the pancreatic head is stained. This part of the pancreas yielded islets of Langerhans rich in pancreatic polypeptide-containing cells and poor in glucagon-containing cells (see Fig. 2, E to H) (×1.3).

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