the data presented in their Table 1, it is apparent that morphine and several endogenous opioid peptides inhibit the nicotinic release of catecholamines from isolated chromaffin cells (approximate ID_{50}s: morphine, $>10^{-4}$ M; β-endorphin, 10^{-6} M; Met-enkephalin, $>10^{-4}$ M).

We have performed similar experiments both on freshly isolated bovine chromaffin cells[2] and on the cells maintained as primary monolayer cultures[3,4] for up to 16 days. We agree with their findings on inhibition of catecholamine release by morphine (ID_{50} 1.6×10^{-4} M), β-endorphin (5×10^{-5} M), Met-enkephalin (5×10^{-4} M) and Leu-enkephalin ($>5\times10^{-4}$ M). Like Costa *et al.*, we found that the nicotine-evoked release but not the K^+-evoked release of catecholamines was inhibited by these compounds[5]. We also agree that the inhibition by these opiate compounds is noncompetitive with nicotinic agonists; however, we are not convinced by the specificity of the inhibitory effects of opioid compounds for the opiate receptor. In our hands, the two opiate antagonists naloxone (10^{-8}–10^{-4} M) and naltrexone (10^{-8}–10^{-4} M) did not reverse the inhibition produced by morphine, but rather they acted like morphine to inhibit the release of catecholamines (naloxone ID_{50}, 10^{-5} M; naltrexone, $>10^{-4}$ M). Further, the effect of naloxone (10^{-6}–10^{-4} M) and naltrexone (10^{-6}–10^{-4} M) was additive to that of β-endorphin (5×10^{-5} M) and morphine (10^{-4} M).

We were rather concerned that the levels of morphine and the endogenous opiates required to inhibit catecholamine secretion (10^{-3}–10^{-5} M) were far in excess of the concentrations of these compounds required for effective receptor occupancy (10^{-8}–10^{-9} M)[1,6]. In our search for compounds with higher potency, we found that levorphanol inhibited catecholamine release with an ID_{50} of 4×10^{-6} M. However, dextrorphan, the inactive enantiomer of levorphanol, was equipotent (ID_{50} 4×10^{-6} M) in inhibiting the release of catecholamine from the chromaffin cells.

It is therefore unlikely that the receptor that is involved in these inhibitory actions of morphine and the endogenous opiates on catecholamine release is the same as that described in membrane preparations from the cells[1] and adrenal medullary homogenates[6] that is stereospecific and has high-affinity binding for Met-enkephalinamide (K_d 0.9×10^{-9} M), naloxone (3.2×10^{-9} M) and naltrexone ($+Na^+$ 1.3×10^{-9} M; $-Na^+$ 3.93×10^{-9} M).

Before any physiological role for opiates in the adrenal gland is considered it is necessary to demonstrate stereospecificity and reversal by antagonists. Although it is possible that slight differences in the way the cells are isolated and maintained in culture could account for the differences we observe, the basic question we raise here of the lack of stereospecificity and the discrepancy between the levels required for receptor occupancy (10^{-9} M) and the biological effects (10^{-5}–10^{-4} M) are still relevant and raise serious doubts about the proposed functional role of high-affinity opiate receptors in the modulation of catecholamine release from chromaffin cells.

SIMON LEMAIRE
IRMA LEMAIRE
Department of Pharmacology,
Centre Hospitalier Universitaire,
Sherbrooke, Quebec,
Canada J1H 5N4

DEANNE M. DEAN
BRUCE G. LIVETT
Division of Neurology,
The Montreal General Hospital and
McGill University,
Montreal, Quebec,
Canada H3G 1A4

1. Kumakura, K., Karoum, F., Guidotti, A. & Costa, E. *Nature* **283**, 489–492 (1980).
2. Schneider, A. S., Herz, R. & Rosenheck, K. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5036–5040 (1977).
3. Livett, B. G., Kozousek, V., Mizobe, F. & Dean, D. M. *Nature* **278**, 256–257 (1979).
4. Mizobe, F., Kozousek, V., Dean, D. M. & Livett, B. G. *Brain Res.* **178**, 555–566 (1979).
5. Mizobe, F., Dean, D. M. & Livett, B. G. *Soc. Neurosci. Abstr.* **5**, 534 (1979).
6. Chavkin, C., Cox, B. M. & Goldstein, A. *Molec. Pharmac.* **15**, 751–753 (1979).

COSTA ET AL. REPLY—Lemaire *et al.* have confirmed our report that opioids, when added to primary cultures of chromaffin cells, inhibit the release of catecholamines elicited by nicotinic receptor agonists. They pointed out that this inhibitory effect is nonspecific because the required dose of morphine is too high and the phenomenon lacks stereospecificity. Moreover, as the doses of naloxone required to reverse the action of morphine are also high, at these doses noloxone acts as a partial agonist. Lemaire *et al.* failed to give significance to our finding that lower concentrations of naloxone are required to antagonize the inhibition of catecholamine release caused by β-endorphin. This peptide seems to have a greater affinity and a greater intrinsic activity with the opiate receptor of chromaffin cells which are operative in regulating the action of nicotinic receptor agonists on chromaffin cell stores of catecholamines. In conclusion, the data obtained by us and confirmed by Lemaire *et al.* show that this opiate receptor has peculiar properties (a low sensitivity to morphine, high sensitivity to β-endorphin). Moreover, as expected, the dose of naloxone required to antagonize the inhibition of catecholamine release elicited by opioids depends on the specificity of the agonist used.

These considerations are in line with current thinking suggesting that there are multiple forms of opiate receptors. Hence, it is not surprising that those located on the membrane of the chromaffin cells have peculiar properties which must be studied before concluding that these receptors do not have a physiological role.

We studied the binding characteristics of the opiate recognition sites of chromaffin cell membranes and compared them with the recognition sites located in synaptic membranes prepared from frontal cortex of bovine brain. The opiate recognition sites of adrenal medulla have a high affinity, and a large capacity to bind etorphine and its structurally related antagonist, diprenorphine. This prompted us to study the action of both compounds on the nicotine-induced release of catecholamines. Diprenorphine, in concentrations of 10^{-8}–10^{-6} M, is devoid of any action, whereas etorphine prevents the action of nicotine with an IC_{50} of 2×10^{-7} M. The IC_{50} of diprenorphine to inhibit the action of an IC_{50} dose of etorphine is about 3×10^{-7} M. These results show a specific ability of etorphine to antagonize nicotine, and a great specificity of diprenorphine to antagonize etorphine.

We believe that the opiate peptides stored in the splanchnic nerve act on specific receptors located on the chromaffin cell membrane. When these peptides are released from the splanchnic nerve they may function as co-transmitters and modulate the number of receptors for acetylcholine that are available in the chromaffin cell membrane. According to this view, the receptor for the co-transmitter, when occupied by a specific agonist, can influence the number of acetylcholine recognition sites and the extent of the response to acetylcholine.

This type of synaptic regulation could be of interest in the development of new neuropharmacological agents that may modify synaptic transmission by acting on co-transmitter receptors. Theoretically, these potential drugs could minimize or maximize the response to primary transmitters without altering the frequency of neuronal activity, because they will not act on the receptor for the primary transmitter and obliterate the transmitter action. Probably, such drugs would not trigger as many side effects as those that block the primary transmitter receptor. The benzodiazepines are a good example of drugs that act on the co-transmitter regulation. By knowing the nature of various co-transmitters operative in different synapses, we might develop specific drugs that, similarly to the benzodiazepines, will modulate synaptic activity and be relatively devoid of untoward side effects.

E. COSTA
A. GUIDOTTI
L. SAIANI
Laboratory of Preclinical Pharmacology,
National Institute of Mental Health,
Saint Elizabeths Hospital,
Washington, DC 20032