depress both muscarinic hyperpolarization[2] and s-i.p.s.ps[9,16]. Indeed, it is not uncommon to find large discrepancies in required concentrations of antagonists tested against applied compared with neurally-released transmitters. As noted[16], this can be explained as due to much greater peak concentrations of transmitter when synaptically released at the receptor sites.

Thus, the more crucial positive evidence for mediation of s-i.p.s.p. by a second transmitter, dopamine, especially in normal intact mammalian sympathetic ganglia (reviewed in refs 2, 10, 12, 16, 17) cannot be ignored and must be dealt with by any alternative hypothesis. On the other hand, the ambiguous pharmacological evidence[1,2,10,16] can be explained on other grounds, and the significance, for transmitter identity, of the reported small increase in g_m (ref. 1) remains to be clarified.

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SHINNICK-GALLAGHER AND COLE REPLY—In our report[1], we provided evidence suggesting that the slow-inhibitory postsynaptic potential (s-i.p.s.p.) in mammalian sympathetic ganglia is due to the monosynaptic activation of muscarinic receptors. Our data did not support Libet's hypothesis of a disynaptic process for the s-i.p.s.p. resulting from muscarinic activation of an interneurone (SIF cell) which releases dopamine[2].

Here we analyse whether acetylcholine (ACh) or dopamine fulfill the six criteria for establishing the identity of the neurotransmitter[3] mediating the s-i.p.s.p.: (1) Localization: ACh is present preganglionically[4] and dopamine fluorescence is observed in SIF cells[5], but whether the SIF cell is an appropriate anatomical substrate for the s-i.p.s.p. is questionable (see ref. 6). (2) Release: ACh is released during presynaptic stimulation[4], but release of ^{3}H-dopamine from sympathetic ganglia has not been demonstrated[6] and loss of the s-i.p.s.p. may occur with no change in dopamine fluorescence[5]. Furthermore, restoration of the s-i.p.s.p. does not depend on extrinsic dopamine[6]. (3), (4) Enzymes: Synthesizing and metabolizing enzymes for ACh are present pre- and postsynaptically[4], respectively, and some dopamine metabolism occurs within the ganglion[4], but this does not necessarily imply a correlation with the s-i.p.s.p. (5) Synaptic mimicry: ACh causes membrane hyperpolarization[1,4,7], having a reversal potential similar to the s-i.p.s.p.[1,8]. We observed small increases in membrane conductance (g_m) during these responses using instantaneous (not steady state) voltage deflections reflecting initial segment spikes, not rectification in the depolarizing direction. Similar small increases in g_m have been reported by others[7,8] and can be detected on close inspection in earlier records[9,10]. Catecholamines do hyperpolarize, but dopamine was the least effective agonist[11,12] and its effects were mediated through α_2-adrenoceptors, not dopaminergic recthermore, restorg1[2]; dopamine seemed an unlikely candidate and was not analysed. (6) Identical pharmacology: Atropine blocks both the s-i.p.s.p. and ACh hyperpolarization[1,6,12] but there is little evidence[13] that α-adrenoreceptor antagonists block the s-i.p.s.p. High concentrations of competitive adrenergic antagonists only slightly depress s-i.p.s.p.[10,13] but do attenuate action potentials[14] and other slow potentials[6,13,14]. Experiments testing whether non-competitive antagonists were more effective at greater concentrations of synaptically released neurotransmitter[13] are inconclusive, as the antagonists inhibit muscarinically mediated responses[15] and bind to muscarinic receptors and calcium channels but have a greater affinity for the calcium channel[16]. We[1,12] and others[6,8,14,17] have found that catecholamine antagonists cause no significant depression of the s-i.p.s.p., but these concentrations of antagonists can completely abolish synaptic responses known to be mediated by catecholamines in other neutrons[18].

Experiments examining the requirement for a second transmitter are neither a criterion nor definitive, because the treatments interfere with membrane mechanisms. We[1] and others[8] observed that an ACh hyperpolarization persisted when the synaptic response, the s-i.p.s.p., was blocked by a Ca^{2+}-free, high-Mg^{2+}, EGTA medium; continued superfusion depressed the ACh hyperpolarization. However, in no other previous reports were those experiments performed on the same neurone or in the same preparation. There is no experimental evidence that the temporal discrepancy could be due to diffusional barriers. On the other hand, muscarinic activation of a Ca^{2+}-dependent K^+ response that persists in Ca^{2+}-free media and is subsequently abolished on repeated application of ACh has been reported in other tissues[19,20]. We observed only that the ACh hyperpolarization persisted in tetrodotoxin (TTX) at the time the synaptic response, the s-i.p.s.p., was blocked[1]. We know of no evidence suggesting that synaptic transmission can occur in the presence of TTX at interneuronal or any known synapse. In another study[7], the ACh hyperpolarization was present, albeit depressed, when the antidromic action potential was abolished. This suggests that TTX may be affecting the membrane mechanism of ACh by blocking resting Na^+ conductance and indirectly affecting the Na^+ pump, intracellular Ca^{2+} concentration and inward Ca^{2+} currents[21]. Similar effects on ACh hyperpolarizations have been observed when extracellular Na^+ was replaced with Li^+ in amphibian sympathetic ganglia[22].

Clearly, the evidence indicates that ACh, not dopamine fulfills all the criteria for the neurotransmitter mediating the s-i.p.s.p. in this[1,8] and other autonomic ganglia[23-25]. There is little basis for a disynaptic hypothesis for the s-i.p.s.p. at any autonomic synapse.

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