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Dopamine as a Synaptic Transmitter and Modulator in Sympathetic Ganglia: A Different Mode of Synaptic Action*

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Abstract. An analysis of the role of adrenergic transmission in mediating the hyperpolarizing, slow inhibitory postsynaptic potential has revealed that dopamine is apparently the specific synaptic transmitter for this response. An additional action of dopamine was discovered, namely the selective facilitation of another synaptic response, the slow excitatory postsynaptic potential. (This potential is a depolarizing response to the muscarinic action of acetylcholine.) This second, modulatory, role of dopamine has characteristics strikingly different from other known modes of synaptic action. After a brief initial action by dopamine, the facilitation of the slow excitatory postsynaptic potential response can persist for hours and is unaffected by a delayed blockade of the postsynaptic receptors for dopamine. This suggests that the modulation consists of a long-lasting metabolic and/or structural change induced in the postsynaptic neuron by dopamine.

These conclusions are based on the demonstrated actions of dopamine and other catecholamines, as well as on effects (on dopamine actions and on slow postsynaptic potentials of alpha-adrenergic blockers, of blockade, of dopamine oxidase, of depletion of ganglionic catecholamine by muscarinic excitation, and of a selective re-uptake of dopamine after such depletion.

Sympathetic ganglia respond to preganglionic impulses with two slow postsynaptic potentials (PSPs) that have synaptic delays in the tens and hundreds of msec and durations in the tens of seconds.[1–3] The mechanisms of electrogenesis of both of the slow PSPs do not involve increases in ionic conductance of the membrane.[4] Their synaptic mediation also differs from that of the well-known fast (excitatory) postsynaptic potential (EPSP). The slow excitatory response (S-EPSP) is elicited by a muscarinic, instead of a nicotinic action, of acetylcholine (ACh).[2,5] The slow inhibitory response (S-IPSP) also involves a muscarinic action by the ACh released from preganglionic terminals; but the evidence strongly supports the hypothesis that this cholinergic action is on an adrenergic interneuron and brings about the release of catecholamine.[2,6] This catecholamine then directly elicits the hyperpolarizing S-IPSP of the ganglion cell. We now present evidence that the specific catecholamine acting as adrenergic transmitter for the S-IPSP is dopamine (3,4-dihydroxyphenylethyl-

amine or 3-hydroxytyramine), rather than norepinephrine or epinephrine. In addition, an extraordinary second action of dopamine was discovered. Dopamine was found to have a persisting facilitatory effect specifically on the slow muscarinic depolarizing response, i.e., on the S-EPSP. Such a long lasting modulating action of one synaptic transmitter on the subsequent electrogenic response to another synaptic transmitter, establishes a different mode of synaptic action that could have great significance for some slow functions of neural systems generally.[6]

Materials and Methods. Superior cervical ganglia of rabbits were employed. Surface recordings were made of potentials of the ganglion with respect to the end of its postganglionic nerve. The chamber for making such recordings in air, with intermittent soaking in various chemical media at 37°C, has been described.[2] For testing more immediate responses to an injection of a dose of a synaptically active substance, recordings were made in a sucrose-gap chamber similar to that described by Kosterlitz *et al.*;[7] experiments with this chamber were all performed at room temperature, about 20°C. Substances to be tested were dissolved in a volume of 0.1 ml and injected into the perfusion input close to the ganglion compartment. This compartment had a volume of about 0.3 ml, and its perfusion flow rate was ordinarily about 0.5 ml per min. All recordings were dc.

Results and Discussion. Purely hyperpolarizing responses were elicited by each of the catecholamines—dopamine, norepinephrine, and epinephrine—with a threshold dose of about 0.1 μg injected into the perfusion current.[6] The response to such a single dose reached a peak in 2–3 min and decayed over an additional 5 min. It has already been shown that this postsynaptic action of a catecholamine has characteristics similar to those of the actual transmitter for the S-IPSP.[5,8] Evidence that dopamine is the specific catecholamine that mediates the physiologically generated S-IPSP will be given below.

Injections of muscarinic agents generally elicited a hyperpolarizing potential followed by a much more prolonged depolarizing one. Such a biphasic response, with surface positive and negative components, is similar to that already described for intra-arterial injections.[9] The two components are expected from our hypothesis on the mediation of the S-IPSP and S-EPSP, respectively. The responses can be elicited by 1 μmol doses of ACh (with its nicotinic action blocked by *d*-tubocurarine at 50 μg/ml), or by muscarinic agents that have little or no nicotinic actions on mammalian tissues, e.g., methacholine (MCh) or bethanechol (BCh).[6] The response to one dose of BCh is seen in Fig. 1. With BCh, the initial hyperpolarizing component is relatively much larger than the depolarizing one; the reverse is true for MCh or ACh, and for subsequent doses of BCh.

Modulation of the depolarizing response to a muscarinic action: After a brief exposure to a small dose of dopamine, the depolarizing responses to tests with the usual doses of muscarinic agent (e.g., MCh) were greatly enhanced for a long time (Fig. 1). This facilitatory action by dopamine has several unique characteristics: (1) The dose of dopamine injected could be so small that it produced no detectable potentials itself (e.g., 0.01–0.1 μg). (2) The facilitatory effect of the single dose of dopamine on subsequent muscarinic depolarizations persisted, sometimes with very little decrement, for two hr or more (Fig. 1); after 0.01 μg of dopamine

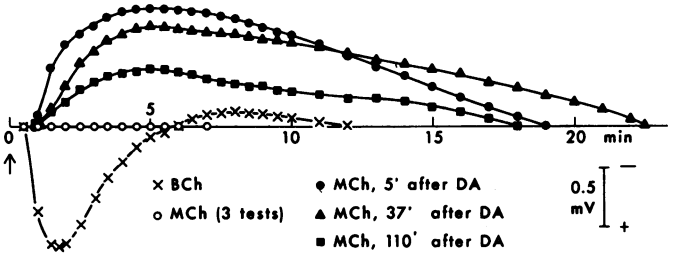

FIG. 1. Muscarinic responses and dopamine facilitation. Surface responses of ganglion, in sucrose-gap perfusion chamber at 20°C, to single dose injections.

x, first response to BCh (bethanechol), 0.5 μmol dose; after this, an additional 9 doses were injected at 3-min intervals to deplete catecholamine stores (see text).

O, response to three separate doses of MCh (methacholine), each 1 μmol, tested at 30-min intervals; all gave no response.

●, MCh (1 μmol) injected 5 min after 1 μg of DA (dopamine); the small hyperpolarizing response to DA itself was completed before the MCh injection.

▲ and ■, MCh tests at 37 and 110 min after the initial, single dose of DA.

the enhancement appeared to decrease progressively over 1 to 2 hr. (3) The facilitatory action of dopamine was effective only on the depolarizing response to muscarinic actions; there was no general or non-specific improvement in cell irritability. There was no effect on the depolarization produced by the nicotinic action of ACh (with atropine present, 20 μg/ml, to block the muscarinic component of ACh action). In accordance with this, the fast EPSPs elicited by orthodromic volleys were also unchanged. (4) Dopamine was by far the most potent of the catecholamines in producing this facilitatory effect on muscarinic depolarizations. The relative ineffectiveness of norepinephrine and epinephrine was most striking when, as in Fig. 1, the control depolarizing (as well as the hyperpolarizing) response to MCh had been reduced to zero; this could be achieved by prior treatments with BCh to produce depletion of stored catecholamine (see below). There was some indication that higher doses of epinephrine (1–10 μg) might have some effectiveness when the control muscarinic response was present at normal levels, but this was difficult to establish definitely.[10]

The unique modulating action of injected dopamine on the muscarinic depolarizing response cannot be regarded as merely of pharmacological interest. Such an action by dopamine appears to occur as a normal and even obligatory part of the synaptic mediation of the S-EPSP that is elicited by preganglionic impulses. This will become evident from the following experiments with blocking agents and with depletion and re-uptake of catecholamines.

Adrenergic blockers: It had been found previously that the "alpha" blocker dibenamine, in low concentrations (1–3 μg/ml) which had relatively little effect on the fast EPSP, could eliminate the S-IPSP response and also severely depress the S-EPSP.[2] Phenoxybenzamine (3 μg/ml) has now been found to be even more selective and much faster in blocking the slow PSPs than is dibenamine, while dihydroergotamine (10-15 μg/ml) was much less effective, and phentolamine had virtually no selective effects. "Beta" adrenergic blockers, such as dichloroisoproterenol(DCI),[2] propranolol, and pronethalol, have been found

to be ineffective. The alpha blockers also abolished the direct hyperpolarizing actions of norepinephrine and epinephrine[11,6] and of dopamine.[6]

The finding that the S-EPSP, a response mediated by a muscarinic action of ACh, could be almost completely eliminated by an adrenergic blocking agent, is now explicable in terms of a blockade of the modulating facilitatory effect of synaptically released dopamine on the S-EPSP. Phenoxybenzamine could in fact completely prevent any facilitatory effect of injected dopamine on the muscarinic depolarization by MCh, when this alpha blocking agent was added to the perfusion fluid some 30 min prior to dopamine. The alpha-type adrenergic receptor for the facilitatory effect is apparently more specialized for dopamine (since the other catecholamines are relatively ineffective) and is presumably different from the receptors that mediate the S-IPSP.

If the lengthy persistence of the facilitation of the S-EPSP by dopamine was due to a durable binding of dopamine to its special receptor sites, then phenoxybenzamine should be able to abolish the facilitation even if administered after dopamine. However, the opposite was found; when phenoxybenzamine was added to the perfusion fluid about 30 min *after* an injection of 1 μg dopamine, the large dopamine facilitation of the depolarizing response to MCh tests (at 30-min intervals) continued for hours thereafter, as usual.[12] This indicates that the facilitatory effect of dopamine involves neuronal changes one or more steps beyond the initial interaction of dopamine with its postsynaptic receptor, since these changes endure independent of any subsequent interference with the dopamine-receptor interaction.

Blockade of dopamine-β-oxidase: Blockade of this enzyme, which converts dopamine to norepinephrine, can be accomplished with diethyldithiocarbamate (DDC)[13]. It tends to increase the intracellular concentration of dopamine while decreasing that of norepinephrine and epinephrine. Addition of DDC (500 μg/ml) to the bathing medium in the air-gap chamber was followed by some enhancement of the S-IPSP and an even more striking enhancement of the S-EPSP (Fig. 2*B*). (The S-IPSP can be seen superimposed on the summated EPSPs during the orthodromic tetanus, as well as continuing into the posttetanic period.) The improvement in the S-IPSP could be demonstrated more clearly when the testing stimulus train (Fig. 2*C*) was preceded by a period of brief conditioning trains which presumably helped to stimulate synthesis or to mobilize

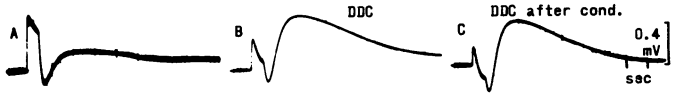

FIG. 2. Effect of DDC (diethyldithiocarbamate) on postsynaptic potentials. Responses in air-gap chamber at 37°C to supramaximal preganglionic stimuli, 40 sec^{-1}, 0.5 sec train duration; ganglion was already sufficiently curarized to prevent firing. Summated EPSP is seen during stimulation (with some superimposed S-IPSP); surface-positive S-IPSP after the 0.5 sec train is followed by longer-lasting S-EPSP.

A, control response. *B*, 30 min after adding DDC, 0.5 mg/ml (some depression of EPSP is a side effect). *C*, same as *B*, but after 20 conditioning trains (each 1 sec, 20 pulses sec^{-1}) of preganglionic stimuli, given at 30-sec intervals.

the transmitter. The actual increase in S-IPSP may in fact have been much greater than it appeared to be in Fig. 2*B* or 2*C*, owing to masking by summation with the greatly enlarged S-EPSP that overlaps temporally with the S-IPSP. In any case, if norepinephrine or epinephrine was the transmitter for the S-IPSP, one would have expected a decrease in the S-IPSP instead of an increase.

The enhancement of the S-EPSP after addition of DDC is explained by the role of dopamine as a modulator of the S-EPSP response. An increase in this facilitatory action apparently results from a rise in the synaptically available amounts of dopamine.

Depletion and re-uptake of dopamine: The presynaptic supply of dopamine should theoretically be decreased by functionally stimulating the adrenergic interneurons to release dopamine excessively. Prolonged treatment (30 min) with the muscarinic agent bethanechol (0.5 mM) should, according to our hypothesis, deplete the adrenergic interneurons at least temporarily of their catecholamine content. Such treatment with BCh in the air-gap chamber (and subsequent washing and recovery for 1 hr) did in fact result in an almost complete disappearance of the S-IPSP (Fig. 3). It also resulted in a more variable but

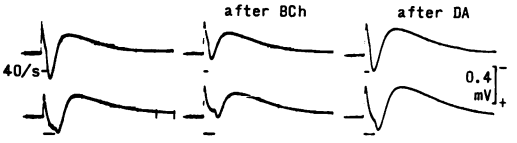

FIG. 3. Depletion by BCh (bethanechol) and re-uptake of DA (dopamine). Recordings and ganglion curarized as for Fig. 2. Responses to 40 sec^{-1} preganglionic stimuli, 0.25 sec trains for top row, 0.7 sec trains for bottom row (see stimulus bars below responses).

"After BCh" responses were obtained after a 30-min incubation with BCh (0.6 mM) in the Ringer solution and a subsequent 60-min period for recovery in Ringer solution without BCh. (Note the almost complete absence now of S-IPSP, and the depressed S-EPSP.) After these tests, dopamine (10 μg/ml) was added, as well as harmine (5 μg/ml) and ascorbic acid (60 μg/ml); this mixture was washed out after 30 min, and the "after DA" responses were obtained 20 min later. Time marks at end of first tracing, bottom row, indicate 1 sec.

always definite reduction in the S-EPSP (Fig. 3); the fast EPSP was unaffected. As already indicated above, the responses to muscarinic agents (e.g., MCh) were also depressed or eliminated (the hyperpolarizing component more readily than the depolarizing one) by repetitive applications of BCh (e.g., Fig. 1). On the other hand, neither the nicotinic depolarization by ACh nor the hyperpolarizing responses to catecholamines were affected.

A crucial test of the validity of the above interpretation, that the effects of prolonged BCh treatment were due to depletion of catecholamines, was to attempt to restore the depressed responses by re-supplying the putatively depleted catecholamines. The bethanechol-treated ganglia were incubated for 30 min with dopamine (10 μg/ml), plus a monoamine oxidase inhibitor (harmine, 5 μg/ml) and ascorbic acid (60 μg/ml), after which the dopamine was washed out. This resulted in a restoration of both the S-IPSP and the S-EPSP (Fig. 3). Even a single small dose of dopamine (1 μg), perfused into the ganglion compartment of the sucrose-gap chamber, could restore the depolarizing action

of MCh for some hours thereafter when given after BCh had abolished the response to MCh (Fig. 1).

The additional important point about the "re-uptake" experiment was that dopamine was the only effective catecholamine. Incubation with norepinephrine produced only a slight restoration of the slow PSPs, and epinephrine produced none. L-dihydroxyphenylalanine (DOPA) was partially effective, as would be expected from its role as a precursor of dopamine.

General Discussion and Conclusions. While all three catecholamines can elicit a hyperpolarizing postsynaptic response that mimics the S-IPSP,[5,8] only dopamine appears to be the one actually released by the presynaptic elements (i.e., by the adrenergic interneurons) that mediate the S-IPSP. This conclusion is based on the effects of the inhibitor of dopamine-β-oxidase, and on the specificity of dopamine in restoring the S-IPSP after the depleting action of bethanechol. Dopamine has been found to be present in sympathetic ganglia in significant amounts.[14,15] Small cells that fluoresce intensely when studied by the technique of Falck and Hillarp for detecting catecholamines, have been found in sympathetic ganglia.[16] These small chromaffin-like granule-containing cells possess the synaptic structures needed for interneurons.[17,18] It has now been reported that the specific catecholamine in these cells is predominantly dopamine.[15] However, the actual release of dopamine, under conditions of orthodromic stimulation adequate for eliciting S-IPSP and S-EPSP, still remains to be investigated.

In the case of the modulating facilitatory adrenergic action on the slow muscarinic depolarizing response that constitutes the S-EPSP, dopamine is by far the most, if not the only, effective catecholamine. Our additional evidence leads to the conclusion that the synaptic release of dopamine is actually involved physiologically in modulating the S-EPSP response to preganglionic impulses, and indeed is a necessary condition for the generation of any S-EPSP. This conclusion is based on the changes in the S-EPSP response that are produced by (1) alpha-adrenergic blockers, (2) the blockade of dopamine-β-oxidase, (3) the apparent depletion of catecholamines after prolonged muscarinic stimulation, and (4) the ability of a re-uptake of dopamine to restore the S-EPSP (and of course the S-IPSP) after such depletion.

The demonstration of a modulating action of one synaptic transmitter on the direct responses to another may serve as a model for a class of synaptic actions and interactions different from those already known. The persisting modulation of the S-EPSP, once it is initiated by dopamine, continues even when there is subsequent interference with the dopamine-receptor interaction. It thus appears to consist of a long-lasting metabolic and/or structural change in the postsynaptic neuron induced by the initial action of the modulating transmitter, dopamine.

Finally, it should be noted that the modulating action described here has several features of potentially great significance for synaptic actions in brain functions. It provides another form of heterosynaptic interaction (i.e., between two different inputs) on the same postsynaptic element. Its capacity for extraordinary persistence for hours, after a single brief exposure to the

modulating transmitter substance, provides a possible mechanism for some of the long-lasting changes exhibited in responses of the brain. Its specific action on an electrogenic process (the S-EPSP) that involves active cell metabolism,[4,6] may provide a route for coupling synaptic actions to long-lasting changes in neuronal metabolism and molecular structure.

Abbreviations used: PSP, postsynaptic potential; EPSP, excitatory (fast) postsynaptic potential; S-EPSP, the slow excitatory response; S-IPSP, the slow inhibitory response; ACh, acetylcholine; MCh, methacholine; BCh, bethanechol; DA, dopamine; DDC, diethyldithiocarbamate; DCI, dichloroisoproterenol.

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[12] Actually it was the peak amplitudes of the depolarizing responses to MCh that were unaffected by the phenoxybenzamine here; the durations of the responses were considerably reduced, i.e., by a faster recovery. The faster recovery is explainable as being due to a weak atropine-like side-action of phenoxybenzamine; the same change can in fact be induced by a weakly effective concentration of atropine. In any case, the striking difference remains between the effects of phenoxybenzamine given before, as opposed to after, dopamine.

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