# Induction and maintenance of ganglionic long-term potentiation require activation of 5-hydroxytryptamine ($5\text{-}HT_3$) receptors

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1. An extracellular recording technique was used to study the effects of 5-hydroxytryptamine (5-HT, serotonin) on the tetanus-induced long-term potentiation (LTP) of the nicotinic pathway of transmission in the superior cervical ganglion (SCG) of the rat. The postganglionic compound action potential (CAP), made submaximal by treatment with hexamethonium (0·4 mM), was used as an index of transmission in the ganglion.

2. Serotonin (10 $\mu$M) markedly enhanced the magnitude of LTP without affecting the post-tetanic potentiation (PTP). The serotonin (2–30 $\mu$M) concentration–response curve for LTP was bell shaped as no enhancement was seen with 30 $\mu$M serotonin. This may largely be due to activation of a $5\text{-}HT_1$ receptor subtype and not to desensitization.

3. When superfused before tetanus, the $5\text{-}HT_{1A}$ receptor agonist 8-hydroxydipropylamino-tetralin (8-OH-DPAT, 5 $\mu$M) prevented the expression of LTP without affecting PTP.

4. Pretreatment of ganglia with the $5\text{-}HT_2$ receptor agonist *R*-(+)-dimethoxy-4-iodo-amphetamine (*R*-(+)-DOI, 1 $\mu$M) enhanced the tetanus-induced LTP. Similar treatment with the $5\text{-}HT_2$ receptor antagonist ketanserin (3 $\mu$M) had no significant effect on LTP.

5. Pretreatment of ganglia with the $5\text{-}HT_3$ receptor agonist 1-*m*-(chlorophenyl)biguanide (*m*-CPBG, 1 $\mu$M), markedly increased (300%) the tetanus-induced LTP. Similar pretreatment with the $5\text{-}HT_3$ receptor antagonist 3-tropanyl-3,5-dichlorobenzoate (MDL 72222, 0·5 $\mu$M) completely prevented the expression of LTP. Fully expressed LTP was reversibly blocked by MDL 72222 when applied during the maintenance phase of LTP.

6. Tetanic stimulation of monoamine-depleted ganglia (from reserpine-pretreated rats, 3 mg $kg^{-1}$ for 24 h) failed to induce LTP.

7. In monoamine-depleted ganglia, tetanus preceded by superfusion with *m*-CPBG readily induced LTP. MDL 72222 completely blocked this LTP. However, in these ganglia tetanus failed to induce LTP when *m*-CPBG was given 2 min (during PTP) or 1 h after tetanus.

8. Tetanic stimulation of monoamine-depleted ganglia in the presence of *R*-(+)-DOI failed to induce LTP.

9. We conclude that tetanus-induced LTP of the SCG of the rat requires activation of $5\text{-}HT_3$ receptors both for induction and maintenance.

Long-term potentiation (LTP) of synaptic transmission is a phenomenon involving use-dependent synaptic plasticity found at many excitatory synapses in the central and peripheral nervous systems. It was first described in the hippocampus (Bliss & Lømo, 1973), but later shown to occur at many synapses in the vertebrate and invertebrate central and peripheral nervous systems. In mammals, most forms of LTP in the central nervous system (CNS) are dependent on activation of the glutamate NMDA receptors. This NMDA-dependent LTP has been so thoroughly investigated that it has somewhat eclipsed the fact that LTP at synapses in the peripheral nervous system and certain regions in the central nervous system is independent of NMDA activation (Johnston, Williams, Jaffe & Gray, 1992). The majority of LTP forms are known to be homosynaptic in that only synapses receiving repetitive stimulation are potentiated.

The presence of use-dependent LTP has been demonstrated in autonomic ganglia of vertebrates. In fact, some of the

earliest reports of long-lasting potentiation came from studies using sympathetic ganglia (Volle, 1966; Dunant & Dolivo, 1968). In the sympathetic ganglia of the bullfrog, tetanic stimulation of the preganglionic nerve induced a long-lasting increase in the quantal content of the excitatory postsynaptic potential (EPSP) in the majority of neurons tested. In some neurons, however, an increase in the quantal size was also seen indicating involvement of the postsynaptic membrane (Koyano, Kuba & Minota, 1985). Similar LTP was discovered in the mammalian sympathetic ganglia (Brown & McAfee, 1982; Briggs, Brown & McAfee, 1985). This LTP is thought to be due solely to an increased release of acetylcholine from the nerve terminals as there was no change in the ACh content of the ganglion (Briggs *et al.* 1985), nor was there a change in the postsynaptic membrane sensitivity to the applied nicotinic receptor agonist 1,1-dimethyl-4-phenyl piperazinium (DMPP; Briggs & McAfee, 1988). More recently, the existence of LTP, strikingly similar to that in mammalian sympathetic ganglia, has been demonstrated in the avian parasympathetic ciliary ganglion (Scott & Bennett, 1993).

In mammalian sympathetic ganglia, the presence of LTP has been demonstrated *in vivo* (Alonso-deFlorida, Morales & Minzoni, 1991; Bachoo & Polosa, 1992), as well as *in vitro* (Brown & McAfee, 1982; Briggs *et al.* 1985; Briggs, McAfee & McCaman, 1988). In the isolated superior cervical ganglion (SCG) of rat (Briggs *et al.* 1985) or guinea-pig (Weinreich, Undem, Taylor & Barry, 1995), a brief tetanic stimulation of the preganglionic nerve can induce LTP manifested as a long-lasting enhancement of the nicotinic pathway. The LTP of the SCG is independent of the activation of either cholinergic or adrenergic receptors. Similar results were reported for the LTP of the avian ciliary ganglion (Scott & Bennett, 1993).

All of these forms of activity-dependent LTP in autonomic ganglia of the vertebrates seem to involve an elevated $Ca^{2+}$ concentration in the presynaptic nerve terminal and are dependent on the presence of $Ca^{2+}$ in the extracellular fluid (Briggs *et al.* 1985; Koyano *et al.* 1985; Scott & Bennett, 1993), yet neither the source nor the trigger for this $Ca^{2+}$ has been identified. Since 5-hydroxytryptamine (5-HT, serotonin) is present in some of the 'interneurons', the small intensely fluorescent (SIF) cells, of the mammalian sympathetic ganglia (Hadjiconstantinou, Potter & Neff, 1982; Happäla, 1988; Paivarinta, Park, Towle & Joh, 1989) we examined the involvement of serotonin in ganglionic LTP.

## METHODS

### Preparation of ganglia

Male Sprague–Dawley rats (200–300 g) were killed by an overdose of pentobarbitone (100 mg $kg^{-1}$ I.P.). Ganglia were rapidly excised and carefully desheathed in oxygenated (95% $O_2$–5% $CO_2$) Locke solution (pH 7·4) containing (mM): NaCl, 136; KCl, 5·6; $CaCl_2$, 2·2; $MgCl_2$, 1·2; $NaH_2PO_4$, 1·2; $NaHCO_3$, 16; and glucose, 11.

**Table 1. Effects of various serotonergic drugs on CAP during post-tetanic (PTP) and long-term (LTP) potentiation**

| | CAP | | |
|---|---|---|---|
| | PTP (% of control) | LTP (% of control) | *n* |
| Untreated | 155 ± 2 | 117 ± 1 | 4 |
| Serotonin (10 μM) | 163 ± 6 | 146 ± 2* | 9 |
| Fluoxetine (10 μM) | 160 ± 7 | 130 ± 2* | 4 |
| 8-OH-DPAT (5 μM) | 173 ± 6 | 109 ± 3* | 4 |
| *R*-(+)-DOI (1 μM) | 167 ± 11 | 140 ± 5* | 4 |
| Ketanserin (3 μM) | 161 ± 2 | 118 ± 5 | 4 |
| MDL 72222 (0·5 μM) | 154 ± 6 | 105 ± 5* | 3 |
| *m*-CPBG (1 μM) | 177 ± 6 | 161 ± 8* | 4 |
| *m*-CPBG + MDL 72222 | 151 ± 3 | 131 ± 2* | 3 |
| Reserpine pretreated rats | | | |
| Control | 152 ± 3 | 102 ± 1* | 7 |
| *m*-CPBG | 153 ± 4 | 135 ± 3* | 4 |
| *R*-(+)-DOI | 151 ± 6 | 102 ± 2* | 4 |

Values are expressed as the mean ± S.E.M. percentage of the CAP recorded immediately before the tetanizing train (Control). For PTP (recorded 2 min after tetanus) there was no significant difference among groups (one-way ANOVA). For LTP (recorded 1 h after tetanus), asterisks indcate significant difference from control value.

### Electrophysiological recording

For recording postganglionic compound action potentials (CAPs), ganglia were placed in a constant temperature (32 ± 1 °C) chamber (3 ml) and the preganglionic (cervical sympathetic) and postganglionic (internal carotid) nerves were gently drawn into capillary stimulating and recording suction electrodes, respectively. The ganglion was continuously superfused with Locke solution at a rate of 1·3 ml $min^{-1}$. The CAPs were evoked by supramaximal stimulation of the preganglionic nerve by 0·3 ms square wave pulses. The CAPs were amplified, displayed on a digital storage oscilloscope and plotted on paper (102XLA oscillograph; Astro-Med, RI, USA) for later measurement. The amplitude of CAP was measured from baseline to spike peak by a computerized digital oscilloscope (model COR 5541U; Kikusui, Kawasaki City, Japan).

### Protocol

After stabilization of the CAP, hexamethonium (0·4 mM) was included in the perfusing Locke solution to partially block the nicotinic pathway in order to obtain submaximal CAPs. This concentration of hexamethonium produces more than 50% reduction in the amplitude of the CAP. Enhanced synaptic efficacy is best evaluated in submaximal postsynaptic responses, but submaximal preganglionic nerve stimulation may result in an increase in recruitment of presynaptic fibres, which may lead to an apparent increase in synaptic efficacy. However, no recruitment was observed when supramaximal stimulation was used (Brown & McAfee, 1992). We therefore used a method modified from Briggs *et al.* (1985) in which submaximal responses were obtained with supramaximal preganglionic nerve stimulation. This was done by

partial blockade by hexamethonium of the response to supramaximal stimulation. An additional period of stabilization of 1 h at the new submaximal amplitude was allowed. During this period, the preganglionic nerve was stimulated once every 5 min. Following this, a tetanizing volley (supramaximal pulses of 0·3 ms duration at 20 Hz for 20 s) was administered. Recordings were made every 2 min for the first 10 min, then every 5 min for the first hour, and finally every 10 min for the next 2 h. Changes in amplitude of CAP are expressed as a percentage of the submaximal (control) CAP recorded immediately before tetanic stimulation. All drugs except reserpine were superfused on the ganglia using a peristaltic pump. In the reserpine series, one dose of reserpine (3 mg $kg^{-1}$ I.P.) was administered to rats 24 h before excision of the ganglia.

### Preparation and sources of drugs

WAY100135 was a gift from Wyeth Research Ltd (Burnham, UK). Other drugs used in this investigation were obtained from Research Biochemicals Inc., except 5-HT hydrochloride and reserpine, which were obtained from Sigma. Stock solutions of drugs were made with distilled water except 3-tropanyl-3,5-dichlorobenzoate (MDL 72222), which was solubilized in 10% HCl. Reserpine solution contained ascorbic acid (0·2% w/v) and sodium metabisulphite (0·1% w/v).

### Statistical analysis

Student's paired and unpaired $t$ tests and one-way ANOVA were employed as appropriate, and are indicated in the text. The minimum level of significance was accepted as $P < 0·05$. Values are given as means ± S.E.M.

## RESULTS

### Effect of serotonin

In the isolated SCG of rat, a brief (20 Hz for 20 s) tetanic stimulation of the preganglionic nerve induces LTP manifested as a long-lasting enhancement of the nicotinic pathway (Fig. 1*B*). This can be measured by recording the postganglionic CAP (Fig. 1*A*). When serotonin (10 μM) was superfused 20 min before tetanic stimulation, it markedly enhanced the tetanus-induced LTP without significantly affecting the basal ganglionic transmission or PTP (Fig. 1*B* and Table 1).

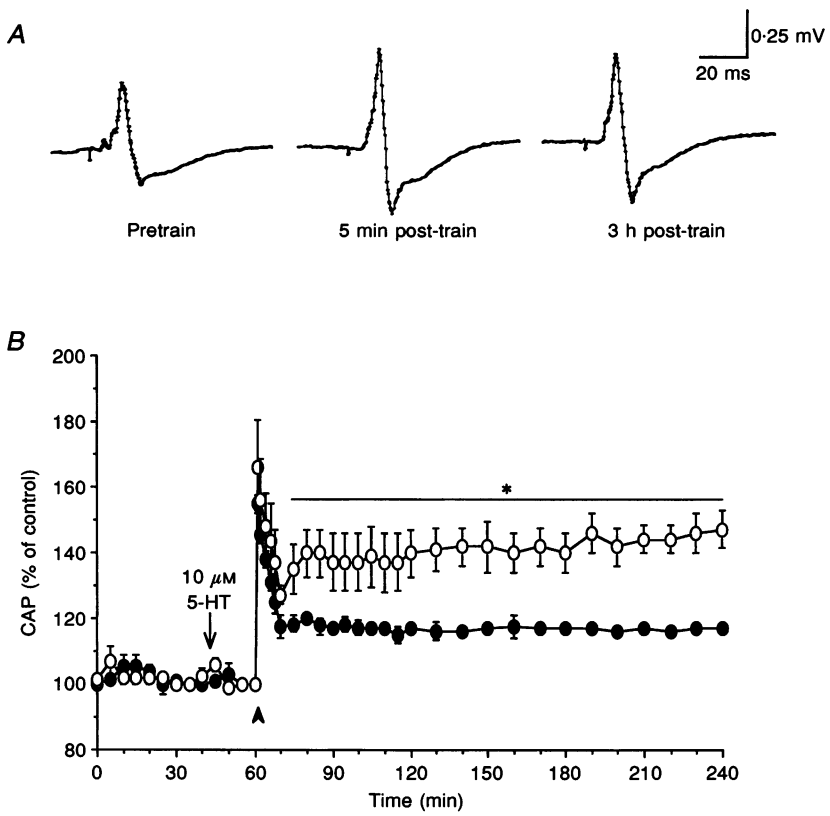


**Figure 1. LTP in the isolated SCG of rat**

*A*, traces of CAPs from a representative untreated ganglion immediately before tetanus (Pretrain), 5 min after tetanus and 3 h later. *B*, the time course of LTP induced by a train of pulses (20 Hz for 20 s, arrowhead) in untreated ganglia (●, mean of 4 ganglia, bars indicate ± S.E.M.). Pretreatment with serotonin (○, arrow) markedly potentiates the LTP (9 ganglia). Asterisk (and underlying bar) indicates those points significantly different (unpaired $t$ test) from the corresponding points from untreated ganglia. The response is expressed as a percentage of control CAP recorded immediately before tetanus. In all ganglia, nicotinic transmission was partially blocked by hexamethonium (0·4 mM) and CAPs were evoked by supramaximal pulses to the preganglionic nerve.

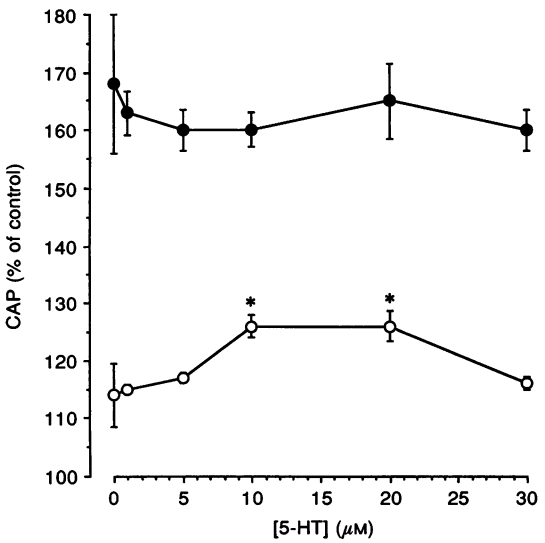


**Figure 2. Concentration–response relationships for the effects of serotonin on PTP and LTP of the isolated SCG of rat**

The magnitudes of PTP (●) and LTP (○) were measured 1 min and 1 h after tetanus, respectively. Both responses are expressed as a percentage of the control response measured immediately before tetanus (3–6 ganglia). Serotonin was superfused 15–30 min before the train. Asterisks indicate a significant difference from values obtained in zero serotonin.

The effect of serotonin on the LTP was studied at various concentrations. Serotonin (1–30 μM), superfused 15–30 min before tetanic stimulation, had no significant effect on the basal submaximal ganglionic transmission or PTP (Fig. 2 and Table 1). Serotonin at 1 or 5 μM did not significantly affect LTP, but at 10 and 20 μM the drug nearly doubled the magnitude of LTP. Higher concentrations reduced the LTP magnitude, and thus a bell-shaped concentration–response curve resulted.

### Effect of fluoxetine

Tetanic stimulation of the preganglionic nerve 30 min after starting superfusion of the uptake blocker fluoxetine (10 μM) resulted in an increase to 130% of control in the amplitude of the CAPs evoked at low frequency after tetanus (Table 1). This represents a 43% increase in the magnitude of LTP produced by the same procedure in the absence of fluoxetine (Fig. 3). Neither the basal ganglionic transmission nor PTP was significantly affected by fluoxetine (Fig. 3 and Table 1).

### Agonist and antagonist of the 5-$HT_{1A}$ receptor

***R*-(+)-8-hydroxydipropylaminotetralin (8-OH-DPAT).** The 5-$HT_{1A}$ receptor agonist 8-OH-DPAT (5 μM), given 15 min before tetanic stimulation, caused a slight inhibition (16 ± 7%, 4 ganglia) of the basal ganglionic transmission. Following tetanus in the presence of the drug, the PTP was basically unaffected; but the LTP was markedly reduced (Fig. 4*A* and Table 1).

**WAY 100135.** From the above result, it follows that blocking 5-$HT_{1A}$ receptors would be expected to enhance LTP. To examine this possibility, the new selective 5-$HT_{1A}$ receptor antagonist WAY 100135 was studied. At 10 μM,

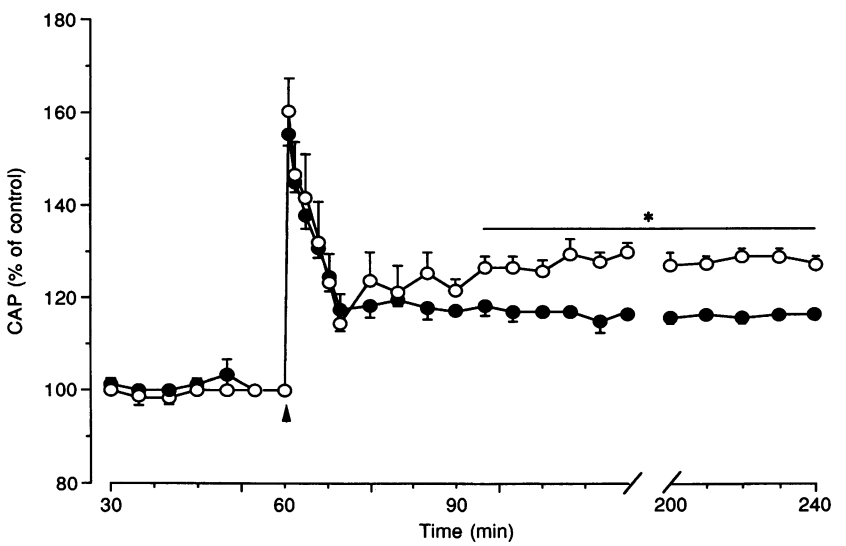


**Figure 3. Pretreatment of ganglia with the serotonin transport inhibitor fluoxetine 30 min before tetanus (arrowhead) enhanced LTP but not PTP**

Results from untreated (●) and fluoxetine-treated (○, 4 ganglia) ganglia. Asterisk indicates significant difference (unpaired *t* test) from corresponding points for untreated ganglia.

WAY 100135 inhibited both PTP and LTP (2 ganglia). Lower concentrations of the drug (0·5 and 1 μM) produced no significant effect on these parameters (6 ganglia, data not shown).

**Agonist and antagonist of the 5-$HT_2$ receptor**

***R*-(+)-2,5-dimethoxy-4-iodoamphetamine (*R*-(+)-DOI).** When superfused on ganglia 20 min before tetanus, the potent 5-$HT_2$ receptor agonist *R*-(+)-DOI hydrochloride (1 μM) caused potentiation of LTP similar to that caused by serotonin itself (Fig. 4*B* and Table 1).

**Ketanserin.** Ganglia were treated with the 5-$HT_2$ receptor antagonist ketanserin. Ketanserin (3 μM) superfusion began 20 min before induction of LTP and did not significantly affect basal ganglionic transmission. The PTP and LTP induced in the presence of ketanserin were not significantly different from the control series (data not shown).

**Agonist and antagonist of 5-$HT_3$ receptor**

**1-(*m*-Chlorophenyl)biguanide (*m*-CPBG).** To further consider the role of the 5-$HT_3$ receptor subtype in LTP, we studied the effect of the selective 5-$HT_3$ receptor agonist *m*-CPBG (1 μM). When superfused before tetanus, it nearly tripled the magnitude of LTP without significantly affecting the basal ganglionic transmission or PTP (Fig. 5 and Table 1). The presence of the 5-$HT_3$ receptor antagonist MDL 72222 (1 μM), markedly attenuated the effect of *m*-CPBG (Fig. 5 and Table 1).

**MDL 72222.** Pretreatment of ganglia with MDL 72222 (0·5 μM) 30 min before tetanic stimulation completely blocked the induction of LTP without affecting the basal ganglionic transmission or PTP (Fig. 6*A* and Table 1). We then examined the role of the 5-$HT_3$ receptor subtype in the maintenance of LTP by superfusing MDL 72222 during established LTP, 30 min (0·5 μM, Fig. 6*B*) or 1 h (1 μM,

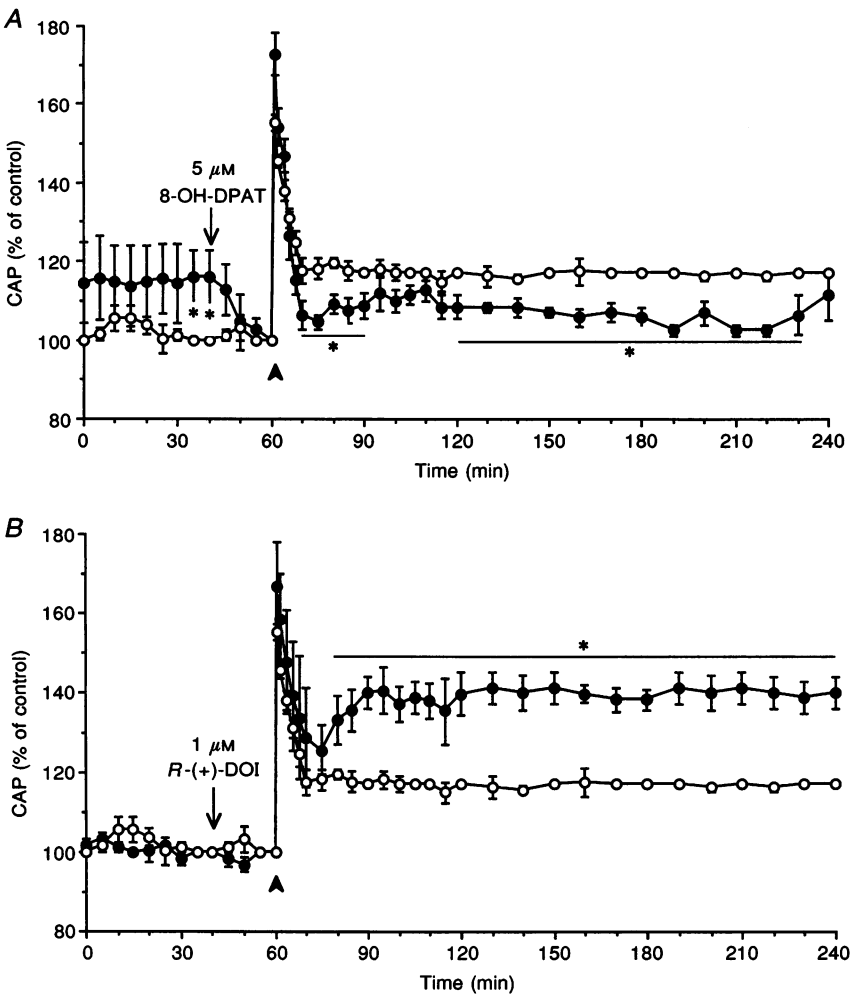


**Figure 4. Effects of 5-$HT_{1A}$ and 5-$HT_2$ receptor agonists on LTP**

*A*, the 5-$HT_{1A}$ receptor agonist 8-OH-DPAT markedly reduced LTP (●). Each point is the mean ± S.E.M. of 4 ganglia. Results from untreated ganglia (○) are shown for comparison. Asterisks indicate a significant difference from corresponding points for untreated ganglia (unpaired *t* test). *B*, the effects of the 5-$HT_2$ receptor agonist *R*-(+)-DOI on the LTP of the SCG of rat (●). Each point represents the mean of 4 ganglia. Results from untreated ganglia (○) are shown for comparison. Asterisk indicates significant difference (unpaired *t* test) from corresponding points for untreated ganglia.

Fig. 6C) after tetanic stimulation. The drug reversibly blocked the established LTP.

### Effect of reserpine

To confirm the role of endogenous serotonin in the induction of LTP we studied ganglia from rats pre-treated with reserpine (3 mg $kg^{-1}$ I.P., 24 h prior to excision of ganglia) to deplete serotonin and other monoamines. In contrast to ganglia from untreated rats, in those from reserpinized (monoamine-depleted) animals tetanic stimulation induced only PTP with no LTP (Fig. 7 and Table 1). This treatment also allowed us to measure the magnitude and time course of PTP uncontaminated by LTP (percentage of control CAP: 1 min after tetanus, $156 \pm 3\%$, $n = 10$; 4 min after tetanus, $104 \pm 2\%$, $n = 14$).

**m-CPBG and MDL 72222.** When m-CPBG (1 μM) was superfused on monoamine-depleted ganglia after tetanic stimulation, the LTP was not induced whether m-CPBG was applied 2 min after tetanus (during PTP, Fig. 7A) or 1 h later (Fig. 7B). In another series of experiments with ganglia from monoamine-depleted animals in which tetanus failed to evoke LTP, a second tetanic stimulation applied 20 min after superfusion of m-CPBG readily induced LTP (Fig. 7C). As long as m-CPBG was present during tetanus in monoamine-depleted ganglia, LTP was fully expressed and maintained (Fig. 8A). The LTP induced in these ganglia in the presence of m-CPBG can be readily blocked by MDL 72222 (Fig. 8B) confirming that 5-$HT_3$ receptor activation is also required for the maintenance of LTP.

**R-(+)-DOI hydrochloride.** Since this 5-$HT_2$ receptor agonist enhanced LTP in normal ganglia (see above), the possibility exists that this receptor subtype may also be involved in the expression of LTP. However, in a series of experiments, pretreatment of monoamine-depleted ganglia with R-(+)-DOI (1 μM) followed by tetanic stimulation failed to induce LTP (Fig. 8C). This indicates that the 5-$HT_2$ receptor is not involved in the induction of LTP.

## DISCUSSION

Our results present compelling evidence showing that activation of 5-$HT_3$ receptors is necessary for the induction, as well as the maintenance, of the LTP in the SCG. Results from monoamine-depleted ganglia exclude the involvement of 5-$HT_2$ subtypes in the induction of LTP.

The role of endogenous ganglionic serotonin in the LTP of the SCG is indicated by the results of experiments with the uptake blocker fluoxetine and by those with the monoamine-depleted ganglia. Fluoxetine consistently enhanced LTP presumably by inhibition of reuptake of released serotonin (Hyttel, 1994). Experiments on monoamine-depleted ganglia unequivocally indicate the absolute requirement for the activation of the 5-$HT_3$ receptor during tetanus to achieve induction and maintenance of LTP. This requirement is indicated by the finding that the 5-$HT_3$ receptor antagonist MDL 72222 consistently blocked the established LTP both in normal and in m-CPBG-treated monoamine-depleted ganglia. In monoamine-depleted ganglia pretreated with m-CPBG, tetanic stimulation induced LTP of a magnitude that is only about 50% of that produced by the same concentration of the drug in normal ganglia. This may further indicate the involvement of endogenous serotonin in the expression of LTP in normal ganglia.

The bell-shaped concentration–response curve of serotonin on the LTP in the SCG is most probably the result of the multiplicity of receptor subtypes that exist in this ganglion.

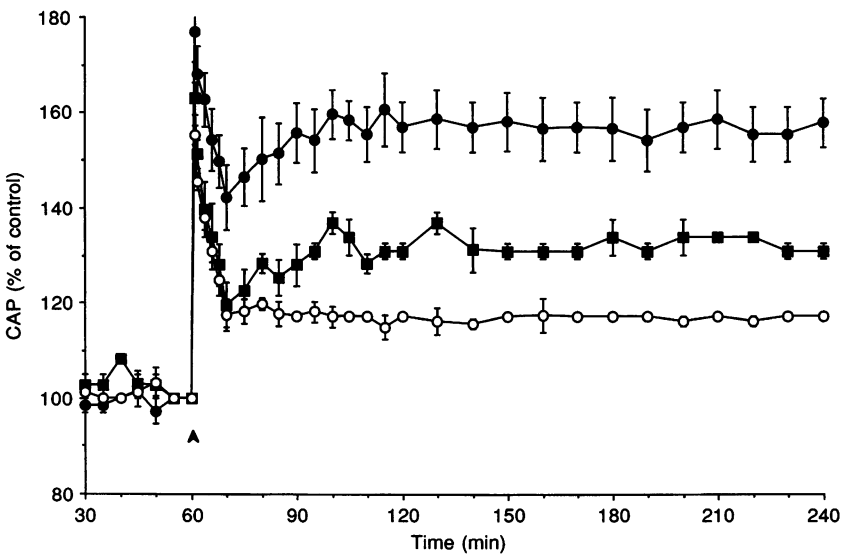


**Figure 5. Effects of the 5-$HT_3$ receptor agonist m-CPBG on LTP**

The 5-$HT_3$ receptor agonist m-CPBG (1 μM, 30 min before tetanus) markedly enhanced the LTP of the isolated SCG of rat (●, 4 ganglia). In the presence of MDL 72222 (■, 4 ganglia), the same concentration of m-CPBG was much less effective. Results from untreated ganglia (○) are shown for comparison.

Activation of the 5-$HT_1$ subtypes in a variety of tissues leads to hyperpolarization and inhibition of neuronal activity (Andrade & Nicoll, 1987; Colino & Halliwell, 1987; Ireland & Jordan, 1987; Salgado & Alkadhi, 1995). The present findings show that pretreatment of ganglia with the 5-$HT_{1A}$ receptor agonist 8-OH-DPAT results in inhibition of basal ganglionic transmission, as well as depression of LTP. The failure of the 5-$HT_{1A}$ receptor antagonist WAY 100135 to enhance LTP probably reflected the possibility that the inhibition was the result of activation of a subtype other than the 5-$HT_{1A}$ receptor. It has recently been reported that inhibition of the CAP of cat sympathetic ganglia was antagonized by a 5-$HT_{1D}$ receptor antagonist but not by a 5-$HT_{1A}$ receptor antagonist (Jones, Martin & Ramage, 1995). In the hippocampus, a similar concentration of WAY 100135 to that used here was sufficient to antagonize the hyperpolarizing effects of serotonin or 8-OH-DPAT on the CA1 pyramidal neurons (Salgado & Alkadhi, 1995). Another possible reason for the bell-shaped curve may be receptor desensitization. However, this is unlikely inasmuch as there was no detectable diminution of the effect of either serotonin or its 5-$HT_3$ receptor agonist *m*-CPBG on the magnitude of LTP for as long as 3 h of superfusion. Moreover, superfusion of high concentrations of serotonin (30 $\mu$M) for 15–30 min before tetanus did not significantly affect the basal CAP.

The 5-$HT_3$ receptors are ligand-gated cation channels and, hence, are structurally and functionally different from all other serotonin receptor subtypes. Electrophysiological studies in sympathetic and visceral primary afferent

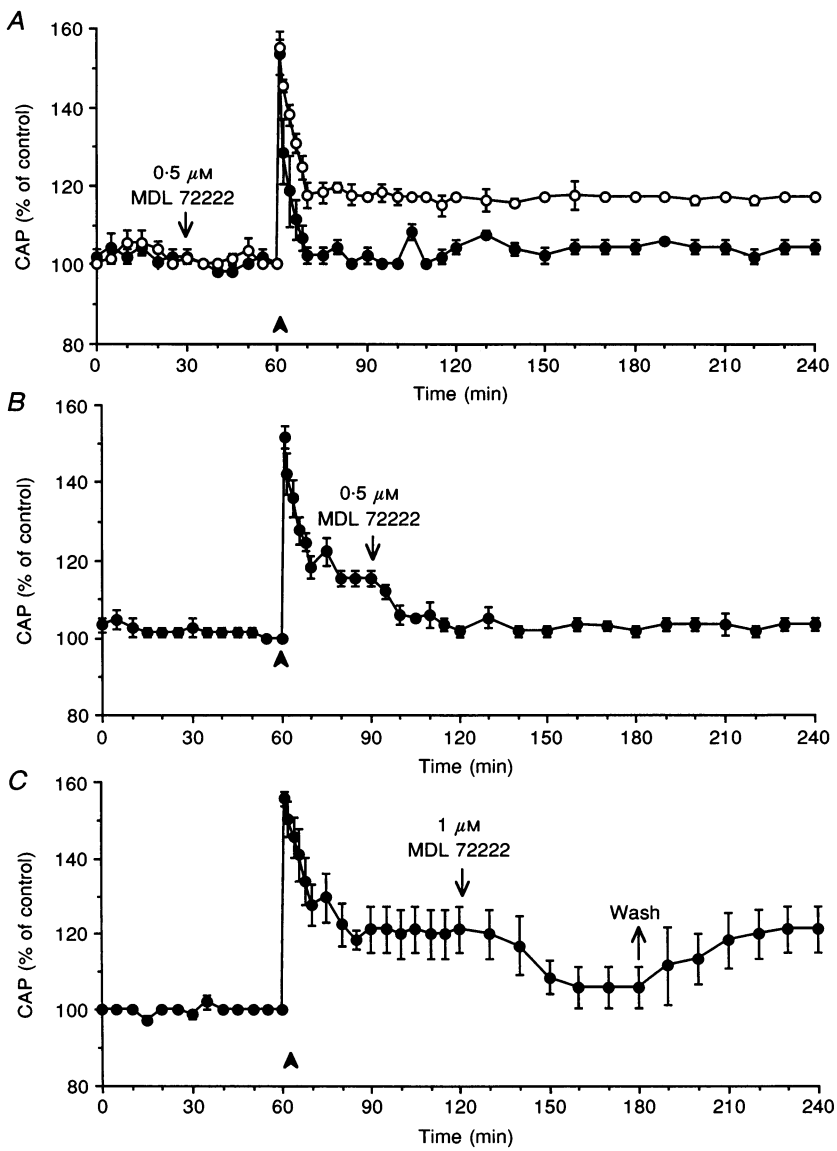


**Figure 6. Effects of 5-$HT_3$ receptor antagonist MDL 72222 on LTP**

*A*, MDL 72222 (●, 4 ganglia) superfused before tetanus markedly blocked the induction of LTP. Results from untreated ganglia (○) are also shown for comparison. MDL 72222, superfused 30 min (*B*, 3 ganglia) or 1 h (*C*, 6 ganglia) after tetanus (arrowhead), reversibly blocked the established LTP.

neurons (Wallis & North, 1978; Higashi & Nishi, 1982) have shown that 5-$HT_3$ receptor activation induces opening of an associated non-specific cationic channel resulting in a simultaneous increase in membrane permeability to sodium and potassium ions and a subsequent rapid influx of extracellular $Ca^{2+}$ (Peters, Malone & Lambert, 1991). This was later confirmed in brain clonal cell lines studied by patch clamp techniques. However, the possibility remains that divalent cations such as $Ca^{2+}$ may also be able to pass through the channel (Lambert, Peters, Hales & Dempster, 1989; Maricq, Peterson, Brake, Myers & Julius, 1991).

Previous work on autonomic ganglia showed that LTP is due to a large increase in $Ca^{2+}$ concentration in the presynaptic nerve terminal, which presumably initiates the cascade for induction of LTP. It is unclear, however, in what manner the activation of the 5-$HT_3$ receptor contributes to this mechanism. One possibility could be that activation of the 5-$HT_3$ receptor provides a precise and focused influx of $Ca^{2+}$ through the associated channel to activate a $Ca^{2+}$-dependent biochemical process, thus starting the cascade for expression of LTP induced by repetitive stimulation. Activation of the 5-$HT_3$ receptors is also needed later for maintenance of the LTP. The exact source of serotonin for both expression and maintenance of LTP is uncertain. One possible source could be the SIF interneurons, but the release of serotonin responsible for LTP has to be independent of activation of

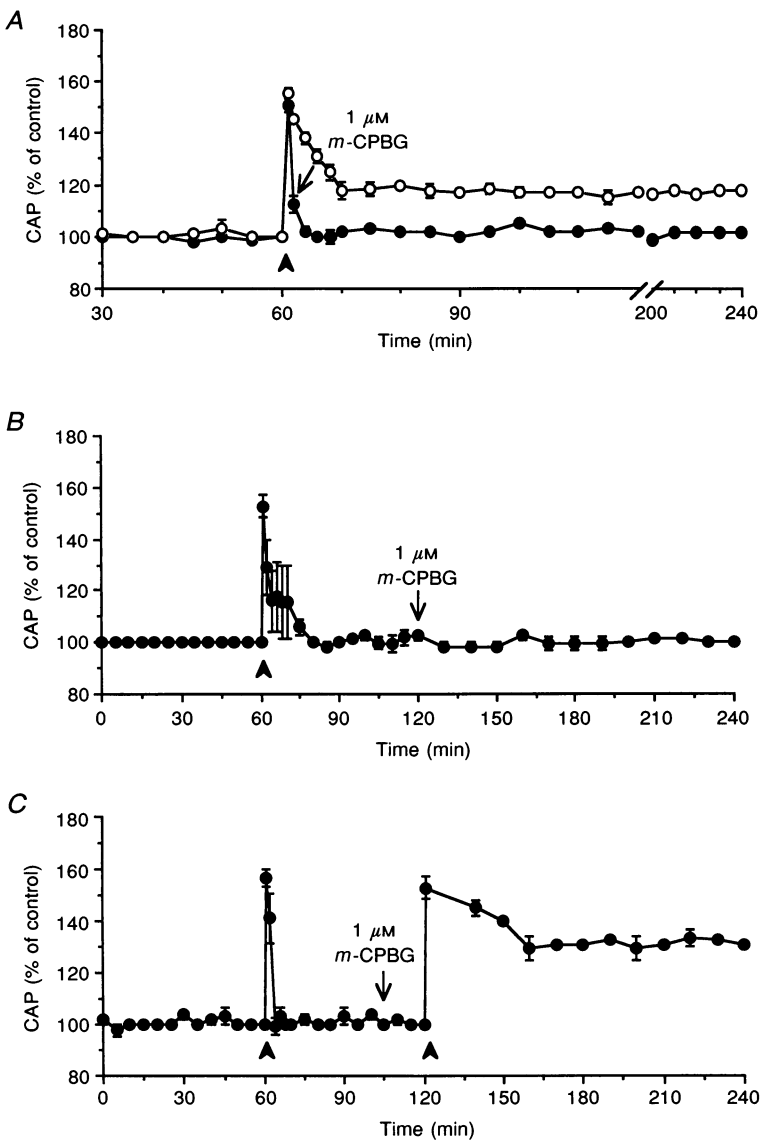


**Figure 7. Effects of reserpine on LTP**

Tetanic stimulation (arrowheads) of monoamine-depleted ganglia from reserpinized animals failed to induce LTP; only PTP was generated (*A*, ●, 4 ganglia). Results from untreated ganglia (○) are shown for comparison. *m*-CPBG had no effect on ganglionic transmission when given during PTP (*A*) or 1 h after tetanus (*B*, 4 ganglia). In another series in monoamine-depleted ganglia, a second tetanus in the presence of *m*-CPBG readily induced LTP (*C*, 4 ganglia). Rats were reserpinized with a single intraperitoneal dose of reserpine (3 mg $kg^{-1}$) given approximately 24 h before excision of ganglia.

nicotinic and muscarinic cholinergic, as well as adrenergic, receptors. This has been determined by Briggs *et al.* (1985), who have shown that LTP in the SCG of rat can be expressed and maintained when all of these receptors are blocked. Based on experiments with MDL 72222, we assume that serotonin is normally released each time the preganglionic nerve is stimulated. In unpotentiated ganglia the released serotonin may have no measurable effect on ganglionic transmission, but in ganglia where LTP has been expressed, serotonin released by single preganglionic pulses would be sufficient to trigger a sensitized process responsible for enhanced acetylcholine release.

A serotonin-dependent LTP is unique in the vertebrate nervous system even though this neurotransmitter is known to modulate various neural responses. In the CA3 neuron of the hippocampus, the LTP in the mossy fibres is inhibited by activation of 5-$HT_3$ receptors. It is suggested that the activation of 5-$HT_3$ may perhaps enhance $GABA_A$ receptor-mediated inhibition (Maeda, Kanelo & Satoh, 1994). Intracellular recordings from the inferior mesenteric ganglion of the guinea-pig showed that serotonin was responsible for the late slow EPSP in some but not all neurons (Wang & Ma, 1990). In the invertebrates, the most significant work in the area of serotonin-dependent synaptic plasticity comes from

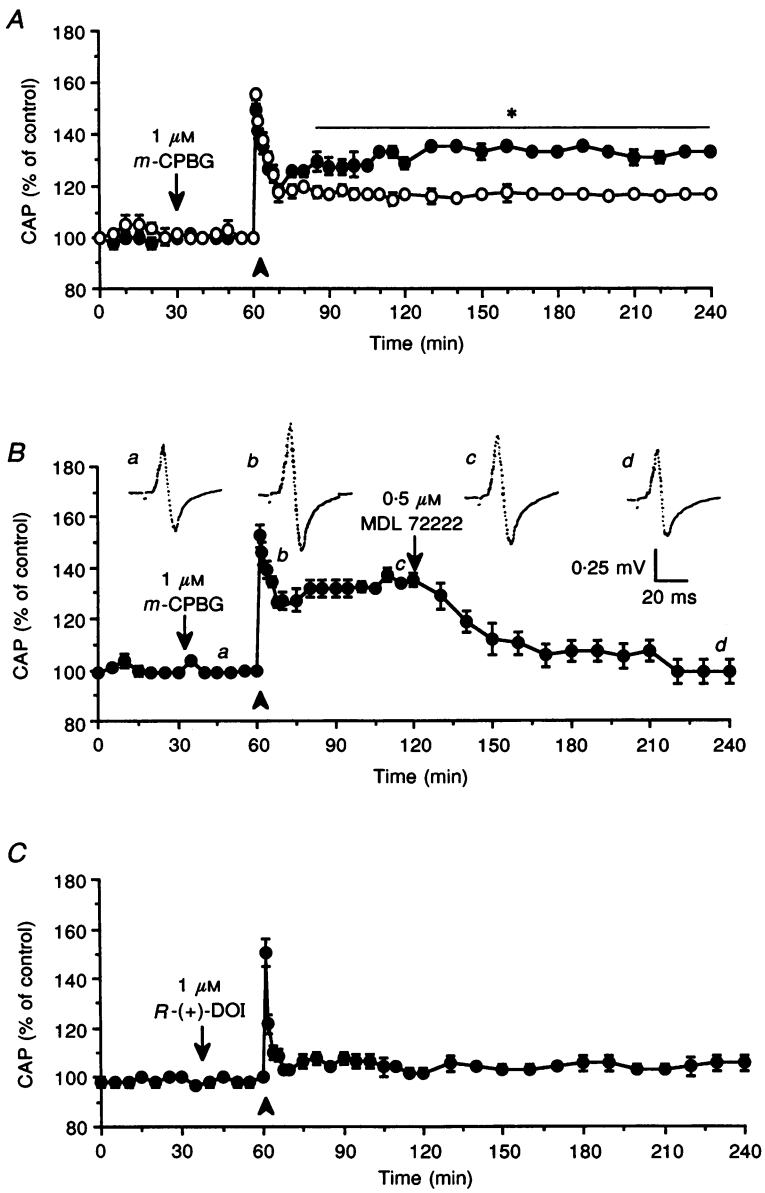


**Figure 8. Effects of serotonin agonists on monoamine-depleted ganglia**

*A*, tetanic stimulation of preganglionic nerve in monoamine-depleted ganglia in the presence of *m*-CPBG produced a robust and long-lasting LTP (●, 4 ganglia). Results from untreated ganglia (○) are shown for comparison (4 ganglia). *B*, the 5-$HT_3$ receptor antagonist MDL 72222 blocked the *m*-CPBG-induced LTP (4 ganglia). Note that in the absence of endogenous serotonin, there was complete antagonism of *m*-CPBG by MDL 72222. Inset in *B*, records of CAPs from one of these ganglia. *C*, in monoamine-depleted ganglia, tetanus in the presence of *R*-(+)-DOI failed to induce LTP (4 ganglia).

*Aplysia*. In this preparation, a use-dependent form of synaptic plasticity causes an LTP-like potentiation of a monosynaptic connection between sensory and motor neurons. Serotonin modulates transmitter release indirectly by increasing $Ca^{2+}$ influx through N-type channels. This occurs as the result of an increase in the duration of the action potential caused by serotonin-induced decrease in $K^+$ current (Baxter & Byrne, 1989; Hochner & Kandel, 1992). However, in *Aplysia*, the serotonin receptor subtype involved in this action is not well defined.

The present results have shown that the induction of LTP in the SCG requires high-frequency stimulation of presynaptic nerve terminals and activation of 5-$HT_3$ receptors simultaneously. Alone, neither of these is sufficient to induce LTP. The LTP of the rat SCG seems to be due to a presynaptic mechanism resulting in potentiation of acetylcholine release with no apparent involvement of the postsynaptic membrane (Briggs *et al.* 1988; Bachoo & Polosa, 1992). Repetitive depolarization of postsynaptic neurons by intracellular current injection failed to induce LTP (Briggs *et al.* 1988). Intracellular recording revealed that changes in the input resistance and membrane potentials of the neuron did not correlate with LTP of the EPSP (Briggs & McAfee, 1988). Furthermore, the response to exogenously applied selective nicotinic receptor agonist DMPP was not increased during LTP (Briggs & McAfee, 1988). Antidromic or intracellular stimulation of ganglionic neurons failed to induce LTP in the rat ganglion (Brown & McAfee, 1982; Briggs *et al.* 1988) even though depolarization should have increased the $Ca^{2+}$ influx through voltage-gated channels. In the bullfrog sympathetic ganglia, tetanic stimulation of preganglionic nerve led to an increase in the quantal content of the EPSP in the majority of neurons. However, in about 50% of these cells there was also an increase in the quantal size suggesting that LTP involves both pre- and postsynaptic regions in the amphibian ganglion (Koyano *et al.* 1985).

Experiments with monoamine-depleted ganglia clearly confirm that LTP and PTP are independent of each other. Although PTP may seem to be enhanced by *m*-CPBG in normal ganglia (Fig. 5 and Table 1), results from monoamine-depleted ganglia indicate that it is not significantly affected by the drug (Fig. 7 and Table 1), and that the apparent increase may be due to overlapping of enhanced LTP. The PTP was not affected by the loss of serotonin and was fully expressed in the absence of LTP. The PTP, a brief increase in the efficacy of synaptic transmission following repetitive stimulation, is also dependent upon extracellular $Ca^{2+}$ concentration (Erulkar & Rahamimoff, 1978). PTP has been demonstrated both in central and peripheral synapses including the hippocampus (Racine & Milgram, 1983), neuromuscular junction (Magleby & Zengel, 1975; Delaney, Zucker & Tank, 1989), sympathetic ganglia and the avian parasympathetic ciliary ganglion (Martin & Pilar, 1964). It is believed that PTP may be due to activation of $Ca^{2+}$ channels during tetanus causing accumulation of $Ca^{2+}$ in the terminal as a result of run-down or saturation of the $Ca^{2+}$ pump (Zucker, 1989). Experiments indicate that $Ca^{2+}$ is elevated in the presynaptic nerve terminal during PTP and the first few minutes of LTP both in the ciliary ganglion (Scott & Bennett, 1993) and at the mossy fibre terminals on CA3 pyramidal neurons (Zalutsky & Nicoll, 1990). This is in contrast to the frog sympathetic ganglion in which similar experiments indicate elevation of intraterminal $Ca^{2+}$ throughout LTP (Minota, Kumamoto, Kitakoga & Kuba, 1991). The status of intraterminal $Ca^{2+}$ concentration during PTP and LTP in the mammalian sympathetic ganglia remains to be studied.


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