Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine that is distributed widely in various body tissues and cell types and possesses a diversity of pharmacological effects at both central and peripheral sites. 5-HT is involved in the control of temperature regulation, cardiovascular function, muscle contraction and endocrine regulation. Peripherally, 5-HT appears to play a major role in platelet homeostasis and the motility of the gastrointestinal tract. Furthermore, 5-HT exerts important effects on skeletal muscles.

**Synthesis and metabolism**

5-HT is synthesized in situ from the amino acid precursor tryptophan. The conversion of tryptophan to 5-hydroxytryptophan is mediated by a tryptophan hydroxylase. 5-Hydroxytryptophan is subsequently decarboxylated to 5-hydroxytryptamine. 5-HT is then metabolized by oxidative deamination by the A enzyme of monoamine oxidase in the liver; the 5-hydroxyindoleacetaldehyde formed from this process is then oxidized to 5-hydroxyindole-3-acetic acid. 5-HT is stored in intracellular vesicles until depolarization of the neurone causes the release of this neurotransmitter into the synapse. Once 5-HT is released into the synapse, its function is terminated rapidly by its presynaptic reuptake into the neurone.

**5-HT receptors and mechanisms of regulation**

Seven major families of 5-HT receptors have been identified, all of which have distinct pharmacological properties, regional distributions and physiological functions. Most of these receptors have several subtypes (e.g. 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$). The physiological effects of 5-HT are mediated by at least four groups of 5-HT receptors, which have been distinguished pharmacologically according to the second messenger systems to which they are coupled. Activation of the first group, including the 5-HT$_1$ and 5-HT$_3$ receptor subtypes, leads to a reduction in adenylate cyclase. Stimulation of the second group of receptors (5-HT$_2$) increases phospholipase C-$eta$/protein kinase C activity. The 5-HT$_3$ receptor is a ligand-gated ion channel and activation of this group, including the 5-HT$_4$, 5-HT$_6$ and 5-HT$_7$ receptor subtypes, leads to increased adenylate cyclase activity. 5-HT receptors belong, with exception of the 5-HT$_3$ receptor, to the gene superfamily of G-protein-coupled receptors. The receptors that interact with G$_s$ proteins stimulate the production of cyclic adenosine monophosphate via adenylate cyclase, whereas those that interact with G$_i$ proteins inhibit the activity of adenylate cyclase. The receptors that interact with the G$_q$ proteins stimulate phosphoinositide hydrolysis via activation of phospholipase C.

**The role of 5-HT in skeletal muscles**

Little is known about the role of 5-HT in skeletal muscle cells. It has been shown that 5-HT induces a concentration-dependent decrease in the rates of alanine and glutamine release from rat muscles in vitro. Furthermore, administration of 5-HT produced an increase in the intracellular concentration of cyclic 3',5'-AMP in these muscle preparations. These data indicate a direct effect of 5-HT on skeletal muscle metabolism via specific receptors and appear to show that the intermediary participation of muscle cyclic AMP is necessary.

Recently, evidence has become available showing that the 5-HT$_{2A}$ receptor is expressed in rat myoblasts and that 5-HT-mediated signalling via this receptor up-regulates the expression of genes involved in myogenic differentiation.
More recently, it was found that adult rat and human muscle expresses the 5-HT$_{2A}$ receptor and that this receptor resides exclusively in plasma membranes and not in transverse tubules. The absence of 5-HT$_{2A}$ receptors in transverse tubule membranes implies that the involvement of this receptor type in excitation and contraction mechanisms is unlikely. Furthermore, it has been shown that 5-HT causes rapid stimulation of glucose uptake into muscle cells, which is mediated by the 5-HT$_{2A}$ receptor.

5-HT and malignant hyperthermia

Malignant hyperthermia (MH) episodes can be triggered in susceptible pigs by environmental stress such as exercise, heating, apprehension and excitement. Previous investigations indicate that the sympathetic nervous system is involved in porcine MH only as a secondary response. Stress significantly increases 5-HT release in the brain and circulating 5-HT levels in the blood. Furthermore, 5-HT exerts direct effects at 5-HT receptors in skeletal muscles. Thus, it has been speculated that specific serotonergic ligands may be involved in mechanisms of stress- and anaesthetic-induced MH.

In vivo studies

The 5-HT$_{2}$ receptor antagonist ketanserin has been shown to be effective in preventing or treating halothane- and stress-induced porcine MH. Without treatment with ketanserin all MH-susceptible animals died. Ketanserin was also shown to be effective in the treatment of stress-induced hyperthermia in North American elks. In this study, 18 elks were allocated randomly to receive either ketanserin or saline after induction of hyperthermia by herding them into distribution chutes and squeeze cages. After herding, the body temperature rose to 43 (1)°C and the animals hyperventilated and had a tachycardia of 111 (10) beats min$^{-1}$. Administration of ketanserin produced significant decreases in temperature and heart and respiratory rates, whereas in the control group values remained increased during the study period. One week later, the experiments were repeated in the same fashion but without any treatment. After this induction of stress, three of nine elks in the ketanserin group died but none of the controls not treated with ketanserin died. The deaths were explained by an excessive temperature increase (more than 45°C in the first experiment), which resulted in greater intolerance of stress during the second study period.

The results with 5-HT$_{2}$ receptor antagonists in the treatment of MH raised the question whether agonists at 5-HT receptors can induce MH. This has been investigated with different 5-HT$_{2}$ receptor agonists. Administration of the 5-HT$_{2}$ receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in MH pigs induced typical signs of MH, including generalized muscle rigidity with subsequent hyperthermia and marked metabolic alterations (Fig. 1).
Furthermore, administration of DOI and the partial agonist d-lysergic acid diethylamide (LSD) produced behavioural changes in conscious pigs, such as grimacing, headshakes and backward locomotion. A few minutes after injection of DOI, the MH animals exhibited mottled skin, cyanosis, hyperventilation and further symptoms of circulatory and metabolic disturbances; three of five pigs died within 60 min. In the surviving animals, the creatine kinase concentration increased from 1000 to 25 000 U litre⁻¹ after 8 h. In normal pigs, stimulation of 5-HT₂ receptors induced transient muscle rigidity and increases in body temperature and ‘psychotic’ behaviour. In contrast to MH pigs, the normal pigs recovered spontaneously after about 3 h. Stimulation of 5-HT₁₄ receptors with specific agonists induced behavioural changes but no signs of MH. This observation suggests that 5-HT₂ receptors are the site of action in 5-HT-induced porcine MH. To investigate whether the effects of 5-HT₂ receptor agonists were mediated through central or peripheral sites of action, the authors tested the in vivo effects of 5-HT, which cannot cross the blood–brain barrier. 5-HT caused mainly vomiting and diarrhoea, but also an increase in body temperature, lactate and creatine kinase levels, indicating a direct effect on skeletal muscle under in vivo conditions.

In further experiments, it was demonstrated that symptoms of MH were reduced or totally prevented by administration of either of the 5-HT₂ receptor antagonists ketanserin and ritanserin.9 These results substantiated the idea that 5-HT₂ receptors are involved primarily in the induction of MH by serotonergic agents. On the other hand, it has been suggested that 5-HT is also involved in the mechanisms of MH induction by volatile anaesthetics.10 Measurements of platelet-free and platelet-rich plasma before and during the onset of porcine MH triggered by halothane showed that physiologically active levels of 5-HT increased concomitantly with the increases in muscle tone, body temperature, venous PCO₂ and plasma lactate. However, it remains unclear whether these changes were induced directly by halothane or were secondary to the development of MH.

Whereas the results of the in vivo experiments with 5-HT₂ receptor agonists clearly demonstrated the involvement of the 5-HT system in MH, studies with 5-HT₂ receptor antagonists revealed inconsistent results. Administration of ketanserin 30 min before halothane completely prevented the induction of MH by a 12-min challenge with halothane in susceptible pigs.7 In contrast to these findings, MH induced by a combination of halothane and succinylcholine in susceptible pigs could not be prevented by pretreatment with ketanserin.11 Ritanserin, a potent antagonist at the 5-HT₂ receptor, was shown to prevent 5-HT-induced MH in pigs.9 However, in later studies ritanserin exerted no therapeutic efficacy on halothane-induced MH in pigs.12 Furthermore, ritanserin was ineffective in preventing anaesthetic-induced MH crises as well as halothane-mediated increases in inositol polyphosphates in MH pigs.13 The reason for the contradictory results of these in vivo experiments remains unclear. Possible explanations include different durations of exposure to the trigger substances and concentrations of halothane, the additional administration of succinylcholine and the use of different breeds of pig.

In vitro studies

In vitro studies showed that the 5-HT₂ receptor agonist DOI induces contractures in skeletal muscle specimens from MH-susceptible (MHS, filled bars) and normal (MHN, open bars) patients after administration of the 5-HT₂ receptor agonist DOI 0.02 mM with and without pretreatment with either ritanserin 0.01 mM for 10 min or dantrolene 1 µM for 10 min. Bars show mean and SEM. *P<0.05 vs normal specimens; †P<0.05 vs DOI; n.c. = no contracture development.

Fig 2 Maximum contracture (mN) in skeletal muscle specimens from MH-susceptible (MHS, filled bars) and normal (MHN, open bars) patients after administration of the 5-HT₂ receptor agonist DOI 0.02 mM with and without pretreatment with either ritanserin 0.01 mM for 10 min or dantrolene 1 µM for 10 min. Bars show mean and SEM. *P<0.05 vs normal specimens; †P<0.05 vs DOI; n.c. = no contracture development.
In subsequent experiments, ritanserin was shown to inhibit halothane-induced contractures in muscles from MH-susceptible patients in a concentration-dependent manner.\textsuperscript{17}

**Inositol polyphosphate system**

The serotonergic effects in MH could possibly be explained by the stimulation of inositol polyphosphate (IP) metabolism via 5-HT receptors in muscle cells. The concentration of inositol-1,4,5-trisphosphate (InsP\textsubscript{3}) within cells is regulated by activation of either G-protein-linked receptors or tyrosine kinase-linked receptors.\textsuperscript{18} External signals and ligands such as 5-HT act through various membrane-spanning receptors which are coupled to phospholipase C through specific G-proteins. After stimulation, phospholipase C hydrolyses phosphatidylinositol 4,5-bisphosphate to give both InsP\textsubscript{3} and diacylglycerol.\textsuperscript{19} The second messenger InsP\textsubscript{3} can stimulate Ca\textsuperscript{2+} release until it is deactivated by rapid dephosphorylation.

It has been proposed that alterations in signal transduction pathways and second messenger systems are intimately involved in MH. Support for this hypothesis was derived from the finding that the InsP\textsubscript{3} receptor shares considerable structural and functional homology with the ryanodine receptor.\textsuperscript{20} Furthermore, the basal concentrations of InsP\textsubscript{3} in skeletal and cardiac muscle of MH-susceptible pigs and humans were found to be significantly elevated.\textsuperscript{18,19} Further investigations have shown significant differences in the threshold for InsP\textsubscript{3}-induced release of Ca\textsuperscript{2+} between MH and normal muscle fibres.\textsuperscript{21} Exposure of MH-susceptible pigs to halothane \textit{in vivo} caused an increase in InsP\textsubscript{3}, whereas there was no effect on IP concentration in normal pigs.\textsuperscript{22} However, ritanserin did not prevent the increase in IP concentration during halothane-induced MH in pigs.\textsuperscript{13} This result indicates that increases in IP concentration during halothane-induced porcine MH might be due to mechanisms other than a 5-HT-mediated increase in IP synthesis. The lack of effect of ritanserin on halothane-induced MH\textsuperscript{12} and the failure to prevent the halothane-induced increase in IP levels\textsuperscript{13} could be related to the high dose of the trigger or to insufficient doses of ritanserin used in the \textit{in vivo} experiments. The latter hypothesis is supported by the fact that the ritanserin doses used for the \textit{in vitro} studies were 2- to 20-fold higher than in the \textit{in vivo} experiments.

**Serotonin syndrome**

The serotonin syndrome is caused by excessive 5-HT availability in the CNS at the 5-HT\textsubscript{1A} receptor. There may also be interactions with dopamine and 5-HT\textsubscript{2} receptors. The serotonin syndrome is most commonly induced by high doses of serotonergic drugs leading to an excessive increase in the intrasynaptic 5-HT concentration.\textsuperscript{23} Serotonin syndrome has also been observed after recent discontinuation of a 5-HT-enhancing drug of long half-life, the effects of which were still present.\textsuperscript{24} Numerous other drugs, including inhibitors of 5-HT metabolism, 5-HT receptor agonists and drugs increasing 5-HT release (e.g. cocaine), induce serotonergic overactivity.

The clinical manifestation of the serotonin syndrome consists of rapid onset of cognitive–behavioural changes, neuromuscular abnormalities and dysfunction of the autonomic nervous system (Table 1). The appearance of a body temperature above 40.5°C usually signals a severe disease process with significant mortality and complication rates.

Treatment of the serotonin syndrome is initiated by discontinuation of serotonergic drugs and in most cases all symptoms disappear within 24 h. In patients with persistent symptoms and/or increasing severity, administration of antiserotonergic drugs to block postsynaptic 5-HT receptors may be considered. Pharmacotherapeutic management also includes benzodiazepines, propanolol and dantrolene. Symptomatic treatment, including cooling, institution of muscle relaxation and mechanical ventilation, may also be required.

<table>
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<tr>
<th>Table 1 Comparison of clinical symptoms of the serotonin syndrome, malignant hyperthermia and neuroleptic malignant syndrome (NMS)</th>
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<td>Symptom</td>
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<td>Rapid onset</td>
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<td>Fever</td>
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<tr>
<td>Muscle rigidity</td>
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<td>Elevated creatine kinase concentration</td>
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<td>Myoglobinuria</td>
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<td>Rhabdomyolysis</td>
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<td>Tachycardia</td>
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<td>Haemodynamic alterations</td>
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<td>Tachyynorea/hypercarbia</td>
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<td>Behavioural changes</td>
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<td>Altered consciousness</td>
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<td>Tremor</td>
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<td>Hyper-reflexia</td>
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Despite the remarkable clinical similarities between serotonin syndrome, neuroleptic malignant syndrome and MH (Table 1), there are important differences in aetiology, clinical course and treatment. MH is induced by the action of volatile anaesthetics and succinylcholine on skeletal muscle cells, whereas serotonin syndrome is induced by the central stimulation of serotonergic receptors and neuroleptic malignant syndrome by blockade of dopamine receptors. The main features of the serotonin syndrome (behavioural changes and altered consciousness) are only rarely observed in MH. In MH the only effective pharmacological treatment is the administration of dantrolene.

Conclusions
The pathophysiological role of the 5-HT system in the regulation of skeletal muscle function and Ca^{2+} release in MH is yet to be defined. Results of in vivo studies in MH-susceptible pigs have shown that agonists at 5-HT receptors are capable of initiating the MH syndrome. MH induced by 5-HT receptor agonists is totally prevented by 5-HT receptor antagonists, but these antagonists were ineffective in the mechanisms of MH in humans. On the basis of the findings of these investigations, it is suggested that a functionally or structurally altered 5-HT system might be involved in the development of MH.

References