Nerve, muscle, and serotonin

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SUMMARY Effects of serotonin on neuromuscular transmission and muscle contraction were studied in the tibialis anterior of rabbits. Serotonin antagonised the Mg^{++} -induced block of transmission, and also provided dual effects on the curare-induced block, anti-curare phase followed by curare-potentiating phase. Independent of transmission processes, serotonin caused a reduction in twitch tension, mainly associated with decreased acceleration of twitch development. These serotonin actions were independent of vascular changes; pharmacological mechanisms are discussed in comparison with those of adrenaline and isoprenaline. A possible role of serotonin in causing a myopathy is proposed.

A relationship between mammalian skeletal muscle and biogenic amine metabolism has been debated in relation to the pathogenesis of human muscular dystrophies. Abnormal contractile response to adrenaline (Takamori, 1975) and impaired activation of adenylyl cyclase by adrenaline (Mawatari *et al.*, 1974) suggest that adrenaline is implicated in abnormalities of the sarcoplasmic reticulum and plasma membrane in dystrophic muscles. Serotonin (5-hydroxytryptamine) is also one of the amines whose role is debated. It has been reported that an experimental myopathy similar to Duchenne muscular dystrophy was produced in the rat by ligation of the abdominal aorta and subsequent administration of serotonin (Mendell *et al.*, 1971, 1972). It was presumed that the pargyline-induced myopathy, a model with histopathological features of Duchenne muscular dystrophy, was caused by an increased level of noradrenaline leading to excessive release of acetylcholine, or to an increased level of serotonin (Yu *et al.*, 1974). Independent of vascular changes, Patten *et al.* (1974) demonstrated serotonin-induced depression of isometric twitch in rat skeletal muscles, and Meltzer (1976) suggested a direct effect of serotonin on rat skeletal muscles in causing myopathy. The present study attempts further to evaluate serotonin actions on neuromuscular transmission and muscle contraction. Investigations were performed in comparison with sympathomimetic amines and their antagonists, the pharmacological actions of which in the neuromuscular system are well known (Bowman and Nott,

1969). Venous outflow from the tested muscle was also determined to ascertain whether or not serotonin effects on the neuromuscular system could be a consequence of vascular changes.

Methods

The experiment was carried out *in vivo* on the tibialis anterior muscle of rabbits weighing from 2 to 2.4 kg, anaesthetised with pentobarbitone sodium (20 mg/kg). The animal was laid on its back and strapped to a heavy plate. The hind-limb on the side to be tested was fixed with a steel pin drilled through the tibia near the knee joint and was taped at the ankle joint to a metal base. The muscle temperature was monitored by a thermocouple inserted into the nearby tibialis anterior and was maintained at 29°C to 30°C. To study the neuromuscular transmission, supra-maximal square electric pulses of 0.1 ms duration were given through an electronic stimulator (Nihon Kohden, MSE-40), isolation transformer (Nihon Kohden, MSE-JH) and platinum wire electrodes hooked onto the peroneal nerve which was surgically exposed and isolated in the popliteal space. The evoked muscle action potential was recorded on an oscilloscope (Nihon Kohden, VC-7) with a plug-in amplifier (Nihon Kohden, AVB-2) and silver surface electrodes (1.5 mm in diameter); with collodion and tape, one electrode was secured over the mid-belly of the tibialis anterior and the other was fixed on its tendon. To study the muscle contraction, the tendon of the tibialis anterior was freed and the muscle was separated from the neighbouring muscles; the tendon was then connected, *via* a stainless steel wire, to a strain gauge and carrier amplifier (Nihon Kohden,

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RM-5). Direct electrical stimulation was applied to the muscle using a bipolar needle electrode inserted under the condition that neuromuscular transmission was blocked by a slow continuous infusion of 0.03% D-tubocurarine chloride through an ear vein. The resting tension was set to obtain maximal isometric twitch and was kept constant. Twitch and tetanus (elicited by 100 Hz repetitive stimulation) were measured on the oscilloscope and these were also transferred to differentiators; the first and second differentials were measured on an ink-writing oscilloscope (Nihon Kohden, W1-130M). The analysis of muscle contraction was done on the basis of the concept of the active state. Measurements and the active state properties were reported elsewhere (Takamori *et al.*, 1971; Takamori, 1975), and are summarised in the Table.

Two types of experimental blockade of the neuromuscular transmission were prepared by means of intravenous injection of 6.7% magnesium sulphate and 0.03% D-tubocurarine chloride through a cannula in an ear vein, respectively. In each experimental state, the drug was given until the amplitude of the muscle action potential evoked by nerve stimulation was reduced to about 50% of the original amplitude; the amplitude was kept at this level by continuous intravenous infusion using a motorised syringe. During the periods of neuromuscular blockades, the following drugs were injected through the cannulated ear vein contralateral to the side of infusion of Mg^{++} or curare: serotonin creatinine sulphate (5 mg/kg), adrenaline hydrochloride (5 μ g/kg), phentolamine mesylate (0.1 mg/kg), propranolol hydrochloride (1 mg), lysergic acid diethylamide (10 mg/kg), dibutyl cyclic AMP (10 mg/kg), sodium caffeine benzoate (50 mg/kg), and calcium gluconate

(225 mg). In the pharmacological study on muscle contraction, serotonin creatinine sulphate (5 mg/kg), lysergic acid diethylamide (10 mg/kg), isoprenaline hydrochloride (5 μ g/kg), and propranolol hydrochloride (1 mg) were injected intravenously. The animal was allowed to breathe spontaneously in all experiments. Using an electromagnetic flowmeter (Nihon Kohden, MS-26) and the method described by Bowman and Zaimis (1958), effects of pharmacological agents on the venous outflow from the tibialis anterior muscle were determined on an ink-writing oscilloscope.

Results

NEUROMUSCULAR TRANSMISSION

Mg^{++} -induced block

The effect of serotonin on a partial neuromuscular block produced by Mg^{++} was tested by measuring the muscle action potential evoked by nerve stimulation at the rate of 0.5 Hz. During the period of 50% block of transmission by Mg^{++} , the intravenous injection of serotonin (5 mg/kg) caused an increase in the amplitude of the evoked muscle action potential which reached its peak (ranging from 57 to 84% of the original amplitude in 12 rabbits) in about two to three minutes, and thereafter gradually waned to the pre-serotonin level (50% of the original amplitude) in about 15 to 20 minutes; this anti- Mg^{++} action of serotonin was prevented by phentolamine (0.1 mg/kg) but not by propranolol (1 mg) and lysergic acid diethylamide (10 mg/kg) (Figs. 1 and 2). This was found to be the same as the anti- Mg^{++} action of adrenaline (5 μ g/kg) which produced an increase in amplitude (57 to 75% of the original amplitude in 11 rabbits) and was abolished only by phentolamine

Fig. 1 Effects of serotonin and adrenaline (injected at bars) on the tibialis anterior muscle action potentials evoked by stimulation of the peroneal nerve at the rate of 0.5 Hz under the 50% neuromuscular block by magnesium infusion. Phentolamine and propranolol were then injected at bars to specify the receptor for each amine.

Fig. 2 Effects of drugs on single evoked muscle action potentials (peroneal nerve–tibialis anterior muscle) during the period of 50% neuromuscular block by continuous infusion of magnesium.

(0.1 mg/kg) (Figs. 1 and 2). The same facilitatory action as serotonin and adrenaline was seen when dibutylrly cyclic AMP (10 mg/kg, 67 and 68% of the original amplitude in two rabbits), caffeine (50 mg/kg, 64 to 87% in five rabbits), and calcium (225 mg, 73 to 80% in four rabbits) were injected during the period of 50% block by Mg^{++} , respectively (Fig. 2).

Curare-induced block

In a partially curarised muscle, as evidenced by 50% reduction in the amplitude of the muscle action potential evoked by nerve stimulation at the rate of 0.32 Hz, the intravenous injection of serotonin (5 mg/kg) caused an anti-curare effect, and this was followed by a prolonged curare-potentiating effect

(Fig. 3). The former ranged from 67 to 80% of the original amplitude in five rabbits, and the latter from 5 to 14% of the original amplitude in five rabbits. Such dual effects of serotonin were found to be the same as those of adrenaline: during the period of 50% block by curare, adrenaline (5 μ g/kg) caused an increase in amplitude (68 to 75% of the original amplitude in three rabbits), and this was followed by a decrease (8 to 10% of the original amplitude in three rabbits) (Fig. 3). In both serotonin and adrenaline effects on neuromuscular transmission, the initial anti-curare action was prevented with pre-treatment by phentolamine (0.1 mg/kg), and the subsequent curare potentiating action was abolished with pre-treatment by propranolol (1 mg). Lysergic acid diethylamide (10 mg/kg) caused no change in serotonin effects on the curare-induced block of transmission. Phentolamine, propranolol, and lysergic acid diethylamide did not themselves affect the amplitude of the evoked muscle action potential in partially curarised muscle.

MUSCLE CONTRACTION

The abbreviations which follow are defined in the Table. In experiments on 15 rabbits anaesthetised by pentobarbitone sodium, the isometric twitch elicited by direct stimulation (under curare block) showed the twitch tension (Pt) ranging from 96 to 140 g, the T_{dPt}/dt from 17 to 18 ms, the $T_{1/2R}$ (decay of the active state) from 45 to 52 ms, and the d^2Pt/dt^2 from 1.4 to 2.1 g/ms². The tetanic force (Po) elicited by 100 Hz repetitive stimulation ranged from 781 to 1197 g, and the dPo/dt ranged from 12 to 15.2 g/ms. Measurements of each abbreviation and their functions in terms of the active state are shown in the Table. The amplitude of simultaneously recorded action potentials ranged from 22 to 26 mV and was equivalent to the amplitude of action potentials evoked by nerve stimulation (without curare block).

Intravenous injection of serotonin (5 mg/kg)

Fig. 3 Effects of serotonin and adrenaline (injected at bars) on the tibialis anterior muscle action potentials evoked by stimulation of the peroneal nerve at the rate of 0.32 Hz under 50% neuromuscular block by curare infusion.

Table *Contractile properties of muscle*

Abbreviations	Measurements	Functions
Pt	Maximum twitch force	Force generated by contractile component plus series-elastic component
T _{1/2R}	Time from onset of twitch development to point of half-maximum tension through to peak of twitch tension	Decay of active state
T _{dPt/dt}	Time from onset of negative deflection of action potential to peak of first differential (Maximum velocity of twitch development)	Duration of active state
d ² Pt/dt ²	Peak of second differential (Maximum acceleration of twitch development)	Active state intensity of shortening
Po	Maximum tetanic force	Active state intensity of load-bearing
dPo/dt	Maximum velocity of tetanus development	Force-velocity relation Rate of formation of cross-bridges Series-elastic component

caused decreases of 11 to 30% in the twitch tension (Pt), which reached the peak 20 to 30 minutes after the injection and then gradually recovered to the pre-serotonin level in about one hour (Figs. 4 and 5). The decrease of Pt by serotonin was accompanied by reduction of the d²Pt/dt² (8 to 19%); changes in the T_{dPt/dt} and T_{1/2R} were none or minimum (Figs. 4 and 5). The decrease of Po by serotonin was slight (0 to 13%) as compared with the decrease of Pt; the decrease of dPo/dt by serotonin ranged from 4 to 14% (Figs. 4 and 5). The muscle action potentials evoked by direct stimulation and nerve stimulation and the resting tension remained unchanged after the injection of serotonin. The twitch-depressant action of lysergic acid diethylamide (10 mg/kg) but not by propranolol (1 mg) and phentolamine (0.1 mg/kg).

As a comparative study, effects of isoprenaline on the tibialis anterior muscle contraction were studied in five rabbits. Intravenous injection of isoprenaline (5 μg/kg) caused increases of 9 to 23% in the twitch tension (Pt) which were accompanied by 10 to 27% prolongation of the T_{1/2R} (Figs. 5 and 6). This twitch potentiation reached the peak five to 10 minutes after the injection and then gradually waned to the pre-isoprenaline level in about 20 minutes. No significant change was found in other properties of the active state. The muscle action potentials evoked by direct stimulation and nerve stimulation and the resting tension remain unchanged after the injection of isoprenaline.

VENOUS OUTFLOW FROM MUSCLE

Aminergic effects on the venous outflow from the tibialis anterior muscle were recorded by means of the electromagnetic flowmeter. Experiments were done in three rabbits for serotonin, adrenaline, noradrenaline,

Fig. 4 *Effects of intravenous injection of serotonin (5 mg/kg) on action potential and active state properties obtained from the tibialis anterior muscle.*

and isoprenaline, respectively; results were confirmed to be consistent in three rabbits for each amine. Serotonin (5 mg/kg) and isoprenaline (5 μg/kg) increased the blood flow, while adrenaline (5 μg/kg) and nora-

Fig. 5 Analysis of mechanisms for the twitch-depressant action of serotonin and for the twitch-potentiating action of isoprenaline on the basis of the active state. Active state properties expressed by these physiological means refer to the Table.

drenaline (5 µg/kg) decreased the flow. The serotonin effect ceased in 18 to 20 minutes and the effects of other amines were abolished within 10 minutes after the intravenous injection (Fig. 7).

Discussion

Magnesium has been known to produce a defect of neuromuscular transmission characterised largely by a decrease in the number of acetylcholine quanta released from the nerve terminal in response to a nerve impulse (Del Castillo and Katz, 1954). Serotonin was found to relieve the Mg⁺⁺-induced block of transmission as manifested by increase in the amplitude of the muscle action potential evoked by nerve stimulation (Figs. 1 and 2), and is thus thought to have a facilitatory action in the acetylcholine quantal release. This is further supported by our finding that the serotonin effect was the same as that of adrenaline, which is known to have a presynaptic action antagon-

ising the Mg⁺⁺-induced block (Figs. 1 and 2) (Krnjević and Miledi, 1958; Jenkinson *et al.*, 1968; Hidaka and Kuriyama, 1969; Kuba, 1970; Singer and Goldberg, 1970). The present result is also consistent with the reports by Dudel (1965) and Dretchen *et al.* (1972) that serotonin acts presynaptically at the neuromuscular junction by increasing the amount of acetylcholine released by nerve stimulation. In this presynaptic action of serotonin, the alpha-adrenergic receptor was indicated to be the receptor as evidenced by the fact that its anti-Mg⁺⁺ effect was prevented by an alpha-adrenergic blocker (phentolamine) but not by a beta-adrenergic blocker (propranolol) and a serotonergic blocker (lysergic acid diethylamide); this was similar to the presynaptic action of adrenaline (Fig. 1) (Bowman and Raper, 1966). The anti-Mg⁺⁺ action common to serotonin and adrenaline was also found with the administrations of dibutyryl cyclic AMP, caffeine, and calcium (Fig. 2). These results suggest that, probably through the serotonin-induced

Fig. 6 Effects of intravenous injection of isoprenaline (5 mg/kg) on action potential and active state properties obtained from the tibialis anterior muscle.

Fig. 7 Effects of amines injected at bars on the venous outflow from the tibialis anterior muscle (rabbit), recorded on ink-writing oscilloscope. Outflow (ml/min) is shown on the ordinate, and the time after injection (min) is shown on the abscissa.

liberation of adrenaline (Douglas *et al.*, 1967), serotonin increases the cyclic AMP content of the nerve terminal and corrects the defect of acetylcholine quantal release by a nerve impulse (Breckenridge *et al.*, 1967; Bowman and Nott, 1969; Rasmussen, 1970; Singer and Goldberg, 1970; Takamori *et al.*, 1973). However, this is contrary in part to the proposal by Dretchen *et al.* (1972) that the presynaptic action of serotonin is mediated by serotonergic receptors.

During the period of the curare-induced block of transmission, serotonin showed dual effects, an initial anti-curare phase followed by a prolonged curare-potentiating phase (Fig. 3). The study using antagonists showed that the former was mediated through the alpha-adrenergic receptor and the latter the beta-adrenergic receptor. Such dual changes in the curarised muscle were also seen after the administration of adrenaline (Fig. 3). The anti-curare action of adrenaline has been attributed to presynaptic facilitatory action in acetylcholine quantal release (Bowman and Raper, 1966); this is similar to the mechanism that we considered in the anti-Mg⁺⁺ action common to adrenaline and serotonin. The curare-potentiating action of adrenaline has been considered to reflect its postsynaptic hyperpolarising action (Bowman and Raper, 1966). Since serotonin showed a curare-potentiating action similar to that of adrenaline and mediated through the beta-adrenergic receptor, this inhibitory effect of serotonin on the curarised muscle is thus suggested to be indirect and due to the serotonin-induced liberation of adrenaline.

(Douglas *et al.*, 1967). The direct action of serotonin on the polarisation of the muscle fibre membrane (Koketsu and Shirasawa, 1974) may also play a role in this postsynaptic effect.

Measuring the isometric twitch, tetanus, and their differentials, the effect of serotonin on muscle contraction was studied on the basis of the concept of the active state (Sandow, 1970; Takamori *et al.*, 1971; Close, 1972; Takamori, 1975). Serotonin caused a reduction in twitch tension (Pt), mainly associated with a decrease in the acceleration of twitch development (d^2Pt/dt^2 , active state intensity of shortening; Table) without change in the evoked muscle action potential (Figs. 4 and 5). The d^2Pt/dt^2 is related to the rate of tension development attained in the very early phase of contraction, and is looked upon as a measure of the amount of calcium released from the sarcoplasmic reticulum in the initiation of excitation-contraction coupling (Sandow *et al.*, 1965; Desmedt and Hainaut, 1968; Taylor *et al.*, 1969). The serotonin-induced reduction of twitch is therefore considered to reflect defects in the subcellular calcium transport system (Ebashi and Endo, 1968). A recent biochemical study reported that repeated injection of serotonin, associated with aortic ligation, caused reduced ability of the sarcoplasmic reticulum to bind calcium in the rat skeletal muscle (Thorpe and Boegman, 1974). Such an effect of serotonin on muscle contraction is in contrast with the well-known effect of isoprenaline on a fast-contracting muscle (Bowman *et al.*, 1962) which, as we confirmed (Figs. 5 and 6), produces an increase in twitch tension (Pt) associated with a prolongation of the $T_{1/2R}$ (decay of the active state; Table) without change of the evoked muscle action potential. The $T_{1/2R}$ reflects the removal of calcium from troponin and the uptake of calcium by the sarcoplasmic reticulum, and may also be related to a relationship between calcium binding by troponin and ATP binding by myosin (Close, 1972). Therefore, serotonin has a twitch-depressant action due to the reduction of the active state intensity of shortening, while isoprenaline has a twitch-potentiating action due to the protracted time course of the active state. The latter has recently been shown to be mediated by the adenyl cyclase-cyclic AMP system of the muscle (Bowman and Nott, 1974). The reduction of tetanic force (Po) after serotonin injection was not so marked (0–13%) in comparison with the reduction of twitch tension (Pt, 11–30%) (Figs. 4 and 5). This measurement expresses the active state intensity of load-bearing (Table) and is related to the number and intrinsic strength of the actin-myosin cross-bridges when the sliding filament mechanism is fully activated (Sandow *et al.*, 1965; Close, 1972). In the study using blocking agents, lysergic acid diethylamide was found to prevent the serotonin-induced reduction of twitch

tension, but neither the alpha-adrenergic blocker nor the beta-adrenergic blocker provided an effect on this serotonin action. These findings support the idea that the twitch-depressant action of serotonin is mediated through its specific receptor of the muscle (Patten *et al.*, 1974).

If serotonin could play a role in causing a myopathy, the present study suggests the following as its possible mechanisms: (1) presynaptic action to release acetylcholine (as evidenced by the anti-Mg⁺⁺ and anti-curare effects), (2) postsynaptic action to alter polarisation of the muscle fibre membrane (as evidenced by the curare-potentiating effect), and (3) inhibitory effect on the subcellular calcium transport system (as evidenced by the twitch-depressant action). Muscle blood flow recording showed that effects of serotonin on neuromuscular transmission and muscle contraction are independent of vascular changes. In neuromuscular transmission, serotonin increased the blood flow through the muscle and adrenaline decreased the flow, but the Mg⁺⁺-induced block was antagonised by both drugs; serotonin had a biphasic action in the curare-induced block as well as adrenaline, but its effect on the blood flow was monophasic (Fig. 7). In muscle contraction, both serotonin and isoprenaline increased the blood flow through the muscle, but effects of these two drugs on the isometric twitch tension were in the opposite direction (Fig. 7). In conformity with this, Dretchen *et al.* (1972) rejected possible effects on the neuromuscular junction mediated through the serotonin-induced increase of blood flow and the serotonin-induced release of potassium. Patten *et al.* (1974) also reported that the serotonin-induced change of blood pressure had no significant effect on twitch tensions. A possible effect of serotonin on muscle temperature was excluded in the present experiment.

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