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Serotonin Depolarizes Type A and C Primary Afferents: an Intracellular Study in Bullfrog Dorsal Root Ganglion

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Abstract

Intracellular recordings were obtained from the somata of type A and C primary afferents in the isolated bullfrog dorsal root ganglion (DRG) preparation. Bath application of serotonin (5-HT) in concentrations of 0.25–1.0 mM led to slow and fast depolarizing responses. Slow, maintained 5-HT depolarizations were observed in 47% of type A and 70% of type C neurons. These slow depolarizations were associated with an underlying increase in input resistance (R_{in}). In some type A neurons, the R_{in} increase was masked by a decrease in R_{in} due to depolarization-induced rectification. The slow 5-HT depolarization of type A, but not type C neurons showed pronounced tachyphylaxis to repeated 5-HT applications. In type C afferents, serotonin's slow action was often accompanied by spontaneous firing. Manganese decreased slow 5-HT depolarizations of both cell types. A slow depolarization and excitation of type C afferents by methysergide and cinanserin was also observed.

Fast transient 5-HT depolarizations accompanied by a rapid decrease in R_{in} were observed in 7% of type A and 24% of type C neurons. In some DRG cells the fast and slow depolarizations combined to form a biphasic response. The actions of 5-HT reported here resemble in some ways 5-HT responses recorded extracellularly from the spinal terminations of primary afferents.

Keywords

serotonin; primary afferent; dorsal root ganglion; methysergide; cinanserin

INTRODUCTION

In vivo studies employing extracellular recording techniques have demonstrated that serotonin (5-HT) excites the peripheral terminations of cutaneous^{2,1}, muscle^{9,25} and visceral²⁷ primary afferent neurons. These actions of 5-HT on sensory nerve endings underlie serotonin's peripheral algescic action^{5,19}. Studies employing isolated nerve and spinal cord preparations have also demonstrated that 5-HT depolarizes and excites the axons²⁶ and central (spinal) terminations of primary afferents^{15,29,35}. This is of particular interest in view of the possibility that endogenously released 5-HT may affect primary afferent transmission in the spinal cord through a direct action on the central terminations of sensory neurons. Analysis of serotonin's actions on primary afferent endings is limited by our inability to reliably impale axons and nerve terminals with microelectrodes for intracellular recording. It is possible, however, to impale and hold the cell bodies of primary afferents^{16,17}. In an intracellular study in the rabbit nodose ganglion, Higashi¹³ reported a 5-

HT depolarization of the cell bodies of unmyelinated (type C) visceral primary afferents. It remains to be determined whether serotonin's actions on visceral afferents are identical to its actions on cutaneous and somatic muscle afferents, the cell bodies of which are located in the dorsal root ganglion (DRG).

As an alternative to intracellular recording, Holz and Anderson¹⁵ employed the sucrose gap technique to obtain extracellular recordings of 5-HT depolarizations of primary afferent cell bodies and central terminations in isolated frog DRG and spinal cord preparations. The similarity of 5-HT responses recorded from DRG and spinal cord preparations suggested that DRG cells may serve as a suitable model of the sensory nerve terminals when studying serotonin's mechanism of action on primary afferents. Employing this model system, we have compared serotonin-induced membrane potential and input resistance changes intracellularly recorded from type A and C somata of bullfrog DRG. Our analysis indicates multiple direct actions of 5-HT on type A and C neurons. Furthermore, the actions of 5-HT on type A and C neurons are not identical, nor are all DRG cells affected.

MATERIALS AND METHODS

Winter and summer bullfrogs (*Rana catesbiana*, 10–15 cm, either sex) were anesthetized in ice and decapitated. The vertebral column with attached spinal nerves was isolated and a laminectomy performed under chilled Ringer solution. Dorsal root ganglia (DRG numbers 9 and 10, nomenclature of Gaupp as described by Kudo²²) with attached dorsal root and spinal nerve were isolated and pinned to a sylgard-lined dish for desheathing. The preparation was transferred to a superfusion chamber (0.25 ml volume) and firmly pinned down. The dorsal root and spinal nerve were passed through vaseline coated slits into adjacent oil pools, mounted on wire electrodes, and stimulated with square wave pulses (0.1–0.2 msec, 0.5–40 V). The distance between the cathode and the DRG was 10 mm.

A limited number of experiments examined serotonin's actions on isolated rabbit DRG and nodose ganglion neurons. Rabbits (white males, 1–3 kg) were killed by air embolism and the DRG or nodose ganglia rapidly removed. These ganglia were prepared for recording as previously described³².

The frog ganglion was totally submerged in Ringer solution of the following composition: NaCl 100.0 mM; KCl, 2.4 mM; NaHCO₃, 9.5 mM; TRIS, 10 mM; CaCl₂, 1.9 mM and dextrose, 5.6 mM. The Ringer solution was saturated with 95% O₂/5% CO₂ (pH 7.4 ± 0.2) and the experiments were performed at room temperature (21–23 °C). The flow rate was 6.5 ml/min. The equilibration time for complete exchange of chamber contents was 20 s and the latency to onset or offset of effect was 3 s as determined by the time course of elevated K⁺ depolarizations.

Intracellular recordings were obtained using 2 M KCl-filled glass microelectrodes (20–80 MΩ). Potentials were amplified and recorded using an amplifier with active bridge circuit allowing current injection through the recording electrode. Current and voltage traces were displayed on a rectilinear pen recorder and digital storage oscilloscope. Input resistance was measured by passing constant current hyperpolarizing pulses of sufficient duration (100–400 ms) to fully charge the membrane capacitance and reach a steady-state voltage deflection. Input resistance measurements were used only if the bridge was well balanced throughout the recording period and was still in balance after the electrode was withdrawn from the cell. The dorsal root or peripheral nerve was stimulated and the latency of indirectly evoked somatic action potentials was measured. The conduction velocity of each cell's axonal process was calculated from the latency measurement and the cell was classified as type A or type C using the classification scheme of Erlanger and Gasser⁸.

Serotonin creatinine-sulfate (Sigma), methysergide maleate (Sandoz) and cinanserin hydrochloride (Squibb) were dissolved in Ringer solution in the desired concentrations and applied in the bath. A valve system was used to switch between control solutions and those containing drugs. Manganese was added to the Ringer solution to block Ca-dependent transmitter release. Manganese was chosen since this ion has been shown to effectively block synaptic transmission in a concentration having little effect on the excitability of axons¹.

RESULTS

Identification of DRG neurons

Stable recordings lasting for up to 6-h duration were obtained from 169 DRG neurons. The distribution of conduction velocities we observed for all DRG neurons is illustrated in the histogram in Fig. 1. The insets in Fig. 1 are examples of indirectly evoked somatic action potentials of type C (left inset) and type A (right inset) neurons. On the basis of conduction velocity (CV) measurements (see Materials and Methods), 120 cells were classified as type A neurons and 49 were classified as type C neurons. The conduction velocities of type A neurons ranged from 4–20 m/s with a mean of 11.5 ± 0.3 m/s (S.E.M., $n = 120$). The conduction velocities of type C neurons ranged from 0.2–0.7 m/s with a mean of 0.47 ± 0.02 m/s ($n = 49$). The threshold stimulus intensity required to activate the axonal processes of type C neurons was 4 to 6-fold greater than that required for type A neurons. The mean input resistance (R_{in}) of type A neurons was 32 ± 3 M Ω ($n = 41$) and the mean resting membrane potential (RMP) was -64 ± 2 mV ($n = 41$). The mean R_{in} of type C neurons was 67 ± 8 M Ω ($n = 13$) and the mean RMP was -61 ± 4 mV ($n = 15$).

Actions of 5-HT on type A neurons

Of 43 type A neurons on which 5-HT was tested, 52% were depolarized; the remaining cells showed no change in RMP. No hyperpolarizing responses were observed. The conduction velocities of serotonin-sensitive neurons ranged from 6–18 m/s. Serotonin depolarizations were of two types: a slowly developing and maintained depolarization noted in 45% of all cells tested (Fig. 2A, top) and a fast transient depolarization of 5% of the cells tested (Fig. 2B). In one case (2%), the fast and slow depolarizations combined to form a biphasic response (Fig. 2C). In many ganglia the slow response to 1 mM 5-HT was only obtained during the first treatment; no subsequent slow depolarizations were observed even after washout periods of several hours. Much less tachyphylaxis was observed for fast transient 5-HT depolarizations which were undiminished in size if repeated at intervals of 10–15 min.

The size of the slow depolarization was dependent on the concentration of 5-HT: the mean amplitude of the response to 1.0 mM 5-HT was 7.3 ± 1.1 mV ($n = 10$), while the mean amplitude of the response to 0.25 mM 5-HT was 4.9 ± 0.6 mV ($n = 7$). Hyperpolarizing current pulses were passed to examine the change in R_{in} accompanying 5-HT responses. Stable determinations of R_{in} were obtained from 19 neurons during slow 5-HT depolarizations. Of these, 58% showed a small decrease in R_{in} at the peak of the response (Fig. 2A, top), 11% showed an increased R_{in} , and 31% showed no change in R_{in} (Fig. 2C). For 1.0 mM 5-HT the mean decrease in R_{in} was $32 \pm 3\%$ ($n = 4$), while for 0.25 mM 5-HT it was $24 \pm 5\%$ ($n = 5$). Stable determinations of R_{in} were also obtained from 2 neurons during fast transient 5-HT depolarizations (Fig. 2B). This depolarization was associated with a rapid decrease in R_{in} which recovered prior to washout of 5-HT. The mean amplitude of the depolarization was 5 mV ($n = 2$) and the mean decrease in R_{in} was 39% ($n = 2$). This response resembled the fast transient 5-HT depolarization of type C neurons described below (Fig. 3B).

As previously reported^{16,34}, the R_{in} of type A neurons was reduced by depolarization. This rectification, shown in Fig. 2C, appears as a non-linearity in the voltage–current (V/I) plot shown in Fig. 2D. The tendency of type A neurons to rectify could explain the decreased R_{in} accompanying slow 5-HT depolarizations. We tested this possibility by passing hyperpolarizing current to manually clamp the membrane potential back to the original resting potential once the peak 5-HT depolarization was attained (Fig. 2C). When artificially repolarized, the voltage deflections produced by hyperpolarizing current pulses were larger than those observed prior to 5-HT. This revealed that the underlying resistance change associated with slow 5-HT depolarization was an increase rather than a decrease.

Although not shown in Fig. 2D, time-dependent (inward-going) anomalous rectification¹¹ similar to that described by Ito¹⁶ was occasionally observed in type A neurons. This rectification was seen with large amplitude (15–40 mV) hyperpolarizing electrotonic potentials.

In the rabbit DRG, 5-HT (0.1–1.0 mM) depolarized type A neurons 3 to 7 mV ($n = 6$) and decreased R_{in} 8 to 57% ($n = 4$). An example is shown in the bottom trace of Fig. 2A. This response resembled the slow depolarization by 5-HT of type A neurons in the frog DRG since the response in both species had similar rise times, decreased R_{in} and exhibited pronounced tachyphylaxis upon repeated administrations. No serotonin-induced firing was observed in type A neurons of frog or rabbit DRG.

Actions of 5-HT on type C neurons

Serotonin (0.1–1.0 mM) was tested on 33 type C neurons. Of these, 76% were depolarized, 21% showed no change in resting potential and one cell (3%) was clearly hyperpolarized. The conduction velocities of serotonin-sensitive neurons ranged from 0.3–0.7 m/s. In general, 5-HT produced larger polarization, R_{in} and excitability changes in type C neurons than in type A neurons. Serotonin depolarizations were of two types: a slowly developing and maintained depolarization of 52% of the cells tested (Fig. 3A and 5B), and a fast transient depolarization of 6% of the cells tested (Fig. 3B, top). In 18% of the cells tested, the fast and slow depolarizations combined to form a biphasic response (Fig. 3C). Unlike serotonin's slow action on type A neurons, the slow 5-HT depolarization of type C neurons showed no tachyphylaxis if intervals of 10–15 min were allowed between applications. If the intervals were less than 10 min, however, the response size was reduced.

An increased R_{in} accompanied slow 5-HT depolarization of type C neurons (Fig. 3A and 5B). Slow 5-HT depolarizations were also accompanied by repetitive firing in 7 of 17 type C neurons tested. For concentrations of 5-HT producing a maximal slow response, the mean amplitude of the depolarization was 11.1 ± 1.0 mV ($n = 31$) and the mean increase in R_{in} was $35 \pm 5\%$ ($n = 20$). We examined whether this increase in R_{in} was secondary to depolarization (anomalous rectification). In two type C neurons the membrane potential was manually clamped back to the original resting potential once the peak 5-HT depolarization and the associated R_{in} increase were reached. Since similar values of R_{in} were measured at the 5-HT depolarized and artificially repolarized levels of membrane potential, the R_{in} increase was not secondary to 5-HT depolarization. This conclusion was supported by the observation that the voltage–current relationship of a serotonin-sensitive type C neuron (Fig. 3D) showed no sign of an increased resistance with depolarization. In fact, large depolarizing current pulses reduced the resistance of type C neurons, although this depolarization-induced rectification was less pronounced than that seen in type A neurons (compare Figs. 3D and 2D). This may explain why the R_{in} increase accompanying slow 5-HT depolarizations was more readily observed in type C neurons than in type A neurons.

Fast transient 5-HT depolarization of type C neurons was associated with a rapid decrease in R_{in} which recovered prior to washout of 5-HT (Fig. 3B, top). This response was occasionally associated with a brief period of firing on the rising phase of the depolarization (not shown). As was the case for serotonin's fast transient action on type A neurons, the fast 5-HT depolarization of type C neurons showed little tachyphylaxis (see above). One case of 5-HT hyperpolarization was observed. This hyperpolarization was associated with a decrease in R_{in} .

In the rabbit nodose ganglion, 5-HT (0.1–0.5 mM) consistently depolarized type C neurons (Fig. 3B, bottom). This depolarization was transient, was associated with a decrease in R_{in} and resembled serotonin's fast action on frog DRG cells.

Effects of manganese on 5-HT responses

Manganese depolarized serotonin-sensitive type C neurons (4 of 5 cases) and type A neurons (4 of 4 cases). In three of 3 type C neurons, Mn^{2+} attenuated but did not block slow 5-HT depolarizations (Fig. 4A, 1–2). Fast transient 5-HT depolarization of type C neurons was also attenuated but not blocked by Mn^{2+} in frog DRG and rabbit nodose ganglion ($n = 2$). In two of 2 type A frog DRG neurons, however, the slow 5-HT depolarization was completely blocked by Mn^{2+} in a reversible fashion (Fig. 4B, 1–3). Note that the R_{in} of the type A neuron was reduced during the Mn^{2+} depolarization (Fig. 4 B2).

Effects of methysergide and cinanserin

Pharmacological studies of serotonin's slow action on type A neurons were limited due to tachyphylaxis. Following application of methysergide (0.5 mM) no 5-HT depolarizations of serotonin-sensitive type A neurons were obtained, even after prolonged (1–2 h) washout of methysergide ($n = 2$). Since weak depolarizing actions of methysergide on type A neurons were sometimes observed, this loss of sensitivity to 5-HT could result from desensitization induced by prolonged methysergide treatment. In contrast, on type C neurons methysergide exhibited strong agonist-like actions (Fig. 5A). This depolarization, with an increase in R_{in} , resembled serotonin's slow action on the same neuron (Fig. 5B). When cinanserin (0.5 mM) was tested on this neuron, it also produced a slow depolarization, an increase in R_{in} and spontaneous repetitive firing (Fig. 5C).

DISCUSSION

In the present study serotonin was found to depolarize primary afferent cell bodies in the frog DRG. One action of 5-HT was a fast transient depolarization of type A and C neurons. A rapid decrease in R_{in} accompanied this response. Higashi and Nishi¹⁴ noted a similar action of 5-HT on the somata of type C visceral afferents in the rabbit nodose ganglion and have proposed that this response results from a simultaneous increase in Na^+ and K^+ conductances.

A second action of 5-HT was a slow depolarization of type A and type C neurons. These depolarizations were associated with an underlying increase in R_{in} which was sometimes masked in type A neurons by a resistance decrease due to depolarization-induced rectification. In type C neurons, serotonin's slow action was often accompanied by spontaneous firing. A slow depolarization and input resistance increase was also observed during exposure to methysergide and cinanserin. Slow 5-HT depolarizations with increased R_{in} and increased spontaneous or evoked firing were not previously reported in the nodose ganglion, but have been observed in the myenteric plexus³⁷, facial motor nucleus³⁶ and in molluscan nervous systems^{6,12,21,28}. These depolarizations may result from a decrease in a

serotonin-sensitive K^+ conductance^{21,33}, an action which may also underlie the slow 5-HT depolarization of DRG cells reported here.

It is possible that 5-HT could indirectly depolarize some primary afferents by releasing depolarizing neurotransmitters from adjacent cells. Such an indirect action is unlikely since previous electron microscope studies failed to demonstrate synapses in the frog DRG²⁴ (but see ref. 18). This conclusion is supported by our observation that a concentration of Mn^{2+} capable of blocking Ca-dependent transmitter release attenuated, but did not block, 5-HT depolarization of type C neurons. Serotonin, therefore, must directly depolarize the somata of primary afferents. Slow 5-HT depolarizations of type A neurons, however, were blocked by Mn^{2+} . This blockade may result, in part, from rectification in response to Mn^{2+} -induced depolarization, as rectification would minimize polarization changes normally associated with serotonin-induced conductance changes.

Some of the actions of 5-HT reported here resemble 5-HT responses recorded extracellularly from the dorsal roots of isolated spinal cord preparations¹⁵. These dorsal root recordings of polarization changes in the central terminations of primary afferents demonstrated fast and slow 5-HT depolarizations due, in part, to serotonin's direct action on afferent nerve endings. As was the case for serotonin's actions on DRG cells, the dorsal root responses exhibited tachyphylaxis to repeated applications of 5-HT and were attenuated by concentrations of Mn^{2+} in excess of that required to block Ca^{2+} -dependent transmitter release. In addition, as was the case for serotonin's excitatory action on type C DRG cells, the exposure of spinal cord preparations to 5-HT led to prolonged repetitive firing originating in afferent nerve terminals. Such similarities between serotonin's actions in DRG and spinal cord preparations suggest that DRG cells may serve as a suitable model of sensory nerve terminals when studying serotonin's mechanism of action on primary afferents.

Our demonstration of serotonin's multiple depolarizing actions on type A and C neurons suggests several roles for 5-HT in the modulation of primary afferent transmission. In the periphery, 5-HT may facilitate transmission by raising the excitability of sensory nerve endings. Prolonged excitatory actions of 5-HT on the peripheral endings of Group II, III and IV cutaneous afferents^{2,10} and Group III and IV muscle afferents^{9,25} have been observed with single fiber extracellular recordings in the cat. In the spinal cord, a role for 5-HT in presynaptic inhibition was previously proposed based on electrophysiological findings^{3,30} and anatomical evidence²³ (but see ref. 31). A fast transient 5-HT depolarization of afferent nerve terminals similar to serotonin's action on DRG cells could result in presynaptic inhibition of primary afferent transmission. The depolarization and associated decrease in R_{in} would attenuate the sensory nerve impulse as it invades the nerve terminal and would reduce voltage-dependent transmitter release⁴. In contrast, facilitation of transmission might result from an action of 5-HT on afferent nerve terminals similar to its slow depolarizing action on type C DRG cells. The increased R_{in} and excitability associated with this depolarization could favor the invasion of afferent terminal arborizations by nerve impulses, thereby increasing transmitter release. It is important to note that 5-HT may also affect transmission through an influence on the Ca^{2+} influx which triggers transmitter release²⁰. Serotonin reduces the calcium component of action potentials recorded from cultured chick DRG cells⁷ and adult frog DRG cells (Holz, unpublished observation). A similar action of 5-HT on the nerve terminals of sensory afferents might inhibit transmitter release. Direct measurement of 5-HT's effect on primary afferent transmitter release will be required to determine the relative importance of these various actions of 5-HT in affecting primary afferent transmission.

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Fig. 1.
The distribution of conduction velocities for 169 frog DRG neurons. Action potentials recorded from DRG somata following peripheral nerve stimulation are shown as insets (arrows indicate stimulus artifacts). Note the difference in spike latency for the type C (CV: 0.4 m/s) and type A (CV: 13 m/s) neurons. The insets have different time bases.

Fig. 2.

Effects of 5-HT on the RMP and R_{in} of type A DRG neurons. Application of 5-HT is indicated by the dark bar below each trace. A: slow 5-HT depolarization with decreased R_{in} . In the top trace, 5-HT (1.0 mM) depolarized this frog type A neuron by 12 mV and decreased R_{in} by 25%. Constant current hyperpolarizing pulses of 0.125 nA were used to monitor R_{in} . Control conditions: RMP, -50 mV; CV, 6 m/s. In the bottom trace, 5-HT (1.0 mM) depolarized this rabbit type A neuron by 6 mV and decreased R_{in} by 43%. Constant current hyperpolarizing pulses of 1 nA were applied. Control conditions: RMP, -64 mV. B: fast transient 5-HT depolarization with decreased R_{in} . Serotonin (0.25 mM) depolarized this frog type A neuron by 4.5 mV and decreased R_{in} by 50%. Hyperpolarizing current pulses of 0.2 nA. Control conditions: CV, 7 m/s. C: biphasic 5-HT depolarization with manual clamp. Serotonin (0.25 mM) produced an initial fast depolarization of 2.5 mV and decreased R_{in} by 25% in this frog type A neuron. The subsequent slowly developing depolarization of 6 mV was accompanied by little or no change in R_{in} . At the peak of the slow 5-HT response, hyperpolarizing current was injected to manually clamp the membrane potential back to the original resting potential. This revealed an increased R_{in} of 67%. Note that injection of depolarizing current prior to the 5-HT response reduced the R_{in} (rectification). Hyperpolarizing current pulses of 0.25 nA were used. Control conditions: RMP, -60 mV; CV, 10 m/s. D: V/I plot obtained prior to 5-HT from a frog DRG type A neuron which responded to 5-HT with a slow depolarization. Note the reduced slope resistance seen with depolarizing current pulses (rectification).

Fig. 3.

Effects of 5-HT on RMP and R_{in} of 5-HT is indicated by the dark bar below each trace A: slow 5-HT depolarization with increased R_{in} and spontaneous firing. Serotonin (1.0 mM) depolarized this frog DRG type C neuron by 22 mV and increased R_{in} by approximately 100%. Action potential amplitudes were attenuated by the pen recorder Hyperpolarizing current pulses of 0.2 nA. Control conditions: RMP, -55 mV; CV, 0.4 m/s. B: fast transient 5-HT depolarization with decreased R_{in} . In the top trace, 5-HT (1.0 mM) depolarized this frog DRG type C neuron by 33 mV and decreased R_{in} by 56%. Hyperpolarizing current pulses of 0.1 nA. Control conditions: CV, 0.3 m/s. In the bottom trace, 5-HT (0.5 mM) depolarized this rabbit nodose ganglion type C neuron by 27 mV and decreased R_{in} by 82%. Hyperpolarizing current pulses of 0.25 nA. Control conditions: RMP, -50 mV; CV, 1 m/s C: biphasic 5-HT depolarization. Serotonin (1.0 mM) produced an initial fast depolarization of 15 mV and decreased R_{in} by 55% in this frog DRG type C neuron. The subsequent slowly developing depolarization of 10 mV was accompanied by an increased R_{in} of 36%. Hyperpolarizing current pulses of 0.2 nA. Control conditions: RMP, -60 mV; CV, 0.5 m/s. D: V/I plot obtained prior to 5-HT application in a type C neuron which responded to 5-HT with slow depolarization and increased R_{in} .

Fig. 4.

Effects of Mn^{2+} on 5-HT responses. A: slow 5-HT (1.0 mM) depolarization of a frog DRG type C neuron prior to (1) and during application of 4 mM Mn^{2+} (2). The dark bars below each trace indicate the duration of exposure to 5-HT and Mn^{2+} . Manganese depolarized this cell and attenuated but did not block the 5-HT response. Control conditions: RMP, -58 mV; CV, 0.3 m/s. B: effects of Mn^{2+} on the 5-HT (0.25 mM) depolarization of a frog DRG type A neuron. In (1) the slow 5-HT response in normal Ringer solution was manually clamped to show an underlying increase in R_{in} at the peak of the depolarization. The amplification was reduced and in (2) the solution was changed to 4 mM Mn^{2+} . Manganese depolarized this cell by 18 mV and decreased R_{in} by 33% (left side of figure). The amplification was increased and 5-HT again tested in Mn^{2+} containing solution. Manganese blocked serotonin's action (right side of figure). Washout of Mn^{2+} repolarized the cell. Recovery of the 5-HT response in normal Ringer solution is shown in (3). Hyperpolarizing current pulses of 0.25 nA in (1), 0.8 nA (left) and 0.4 nA (right) in (2) and 0.4 nA in (3). Control conditions: RMP, -57 mV; CV, 13 m/s.

Fig. 5.

Effects of methysergide, 5-HT and cinanserin on the same type C neuron. The dark bars below each trace indicate the duration of exposure to each agent. A: methysergide (0.5 mM) slowly depolarized this cell by 17 mV and increased R_{in} by 73%. B: the cell was next treated with 5-HT (0.5 mM) which also led to a slow depolarization of 18 mV and increased R_{in} by 45%. C: cinanserin (0.5 mM) slowly depolarized this cell by 24 mV and increased R_{in} by 67%. Note the spontaneous repetitive firing on the rising phase of the cinanserin depolarization. During washout, the time base was expanded (at arrow) and anode break excitation observed. Hyperpolarizing current pulses were 0.12 nA in (1) and 0.07 nA in (2) and (3). Control conditions: RMP, -80 mV; CV, 0.4 m/s.