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THE OTHER (MUSCARINIC) ACETYLCHOLINE RECEPTORS IN SYMPATHETIC GANGLIA: ACTIONS AND MECHANISMS

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Acetylcholine released from preganglionic sympathetic fibers can activate two types of acetylcholine receptors in sympathetic neurons, nicotinic and muscarinic. The former are ligand-gated ion channels responsible for direct synaptic transmission; the latter are G protein-coupled receptors that mediate various indirect modulatory effects. Most mammalian sympathetic neurons express three muscarinic receptor subtypes, M1, M2, and M4; some also express M3 receptors. Activation of M1 receptors stimulates the G protein Gq and causes a slow postsynaptic depolarization and an increase in the excitability, ultimately leading to an asynchronous action potential discharge, which can "break through" the nicotinic ganglion block. This is largely mediated by closure of voltage-gated K^+ channels (the M channels) composed of Kv7.2 and Kv7.3 subunits and results from hydrolysis and depletion of membrane phosphatidylinositol-4,5-bisphosphate. Activation of M2 receptors hyperpolarizes and inhibits the postsynaptic neuron by opening G protein-gated inwardly-rectifying Kir K^+ channels via the G protein Gi. M4 receptors inhibit N-type (CaV(2)) calcium channels via the G protein Go. In the postganglionic neuron somata, this enhances the excitability by reducing calcium-dependent potassium currents. Conversely, in postganglionic processes and axon terminals, CaV(2)-mediated inhibition reduces norepinephrine release and inhibits postganglionic transmission. Different muscarinic receptors may be anatomically segregated with their cognate G proteins and (in some cases) ion channels in signalling microdomains.

Keywords: acetylcholine, muscarinic receptors, ion channels, G protein, phosphatidylinositol-4,5-bisphosphate, microdomain.

In his pioneering studies on transmission through the sympathetic ganglia, Vladimir Skok concentrated on the interaction of acetylcholine (ACh) with nicotinic receptors (nAChRs) – not unreasonably, because activation of released ACh with the subsynaptic nAChRs is the basis for normal fast transmission through the ganglion. "Spillover" of ACh released from preganglionic fibers can, however, also activate muscarinic acetylcholine receptors (mAChRs). Thus, as is shown in Fig. 1, the normal nAChR-driven fast excitatory postsynaptic current (fast EPSC) is followed some 250 msec later (C) by a slower mAChR-driven (atropine-sensitive) postsynaptic current, the slow EPSC (B), as first reported by Libet, Koketsu, Nishi and colleagues (see [1]). Though constituting only about 5% of the fast EPSC amplitude after a single presynaptic shock, it becomes more prominent after tetanic stimulation (B) or after inhibiting acetylcholinesterase (AChE); the consequent slow depolarization can then induce a sustained asynchronous postganglionic discharge (D) seen *in vivo* as a postganglionic after-discharge [2]. One important physiological consequence is the induction of an mAChR-mediated "breakthrough" of the nicotinic receptor transmission block at high frequencies of preganglionic stimulation (e.g., [3.4]; Fig. 2).

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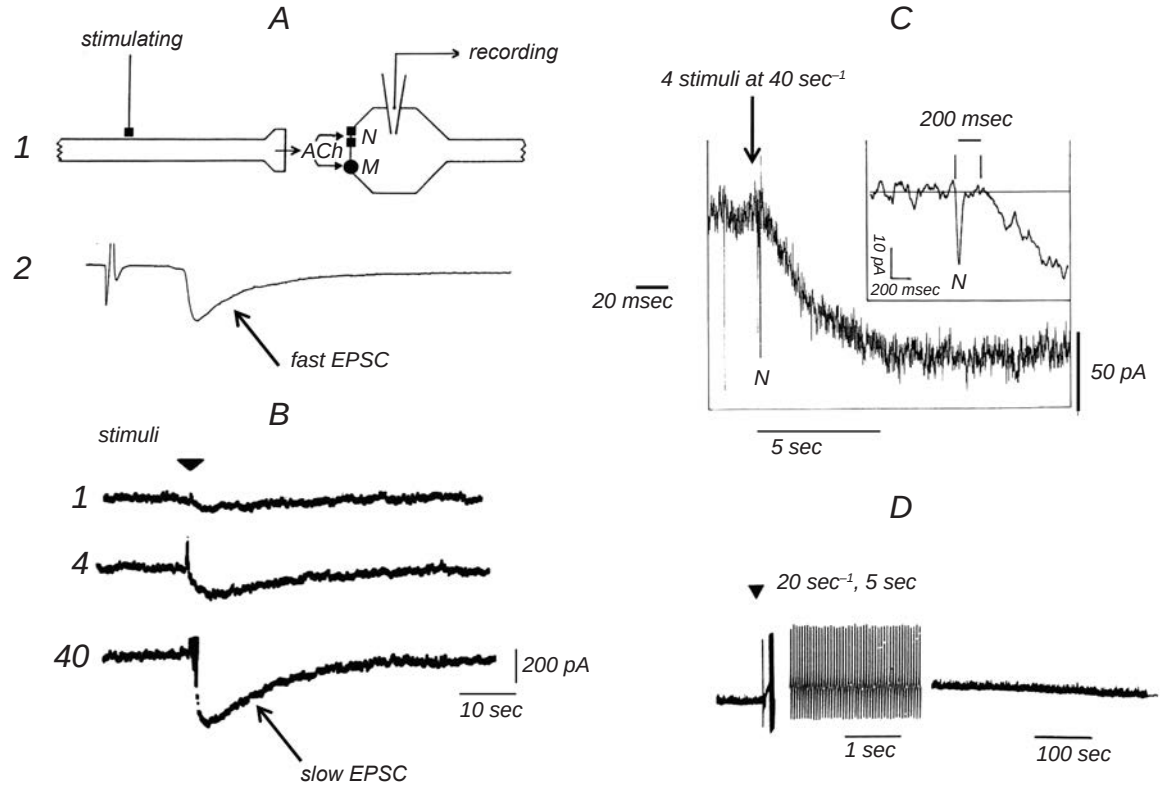
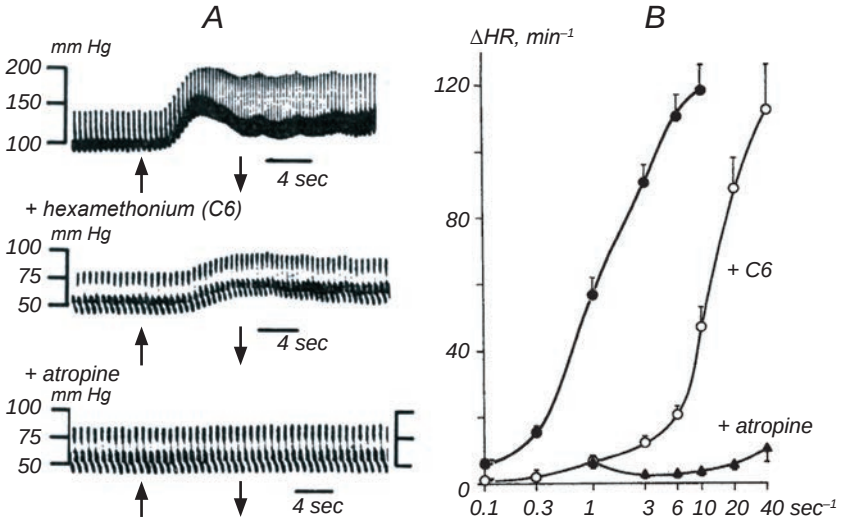


F i g. 1. Nicotinic (N) and muscarinic (M) transmission in the rat superior cervical ganglion. A**)** Microelectrode recording from a single neuron in the isolated intact rat sympathetic superior cervical ganglion (SCG) after stimulation of the preganglionic nerve trunk. 1) Scheme; 2) a single preganglionic stimulus produces a short-latency fast excitatory postsynaptic current (fast EPSP). B**)** Nicotinic receptors are blocked with 100 μM d-tubocurarine (dTC). Preganglionic stimulation now induces a smaller longer-lasting inward current (the slow EPSC); the amplitude of the latter increases with increasing number of stimuli (shown at the left; at 40 sec^{-1}). This can be fully blocked by 1 μM atropine (not shown). C) Slow EPSC recorded in the presence of 100 μM dTC and 1 μM neostigmine (at 34°C). The current builds up over several seconds, with a latency to onset (measured from the residual fast EPSC marked N) of 260 msec (about 2 sec at 24°C). D) Under the same conditions as in C, preganglionic tetanus produces a slow sustained depolarization and a prolonged ganglion cell action potential discharge. (Note that the recorder was speeded up ×100 for 2.5 sec during the discharge). Data of A, B, C, and D are from [5], [6], [7], and [5], respectively.

Р и с. 1. Нікотин (N-) та мускаринергічна (M-) передача у верхньому шийному ганглії щура.



F i g. 2. Muscarinic "breakthrough" of nicotinic ganglion block in anaesthetized dogs. A) Blood pressure responses (mm Hg) to stimulation of the descending thoracic sympathetic postganglionic trunk at 20 sec^{-1} for the period indicated by arrows. Hexamethonium chloride infused i.v at 50-200 mg/kg·hr, atropine injected i.v at 0.03 mg/kg. B) Changes in the heart rate (DHR, ordinate, min^{-1}) to stimulation of the cardiac preganglionic nerves for 30 sec at the frequencies indicated (abscissa, sec^{-1}). Hexamehonium (C6), 10-30 mg/kg, and atropine, 0.03 mg/kg. Note that in both cases atropine blocks the residual hexamethonium-insensitive response. Data in A, adapted from [3]; those in B, from [4].

Р и с. 2. Мускариновий „прорив" нікотинового блокування трансгангліонарної передачі у наркотизованих собак.

MUSCARINIC RECEPTORS (mAChRs)

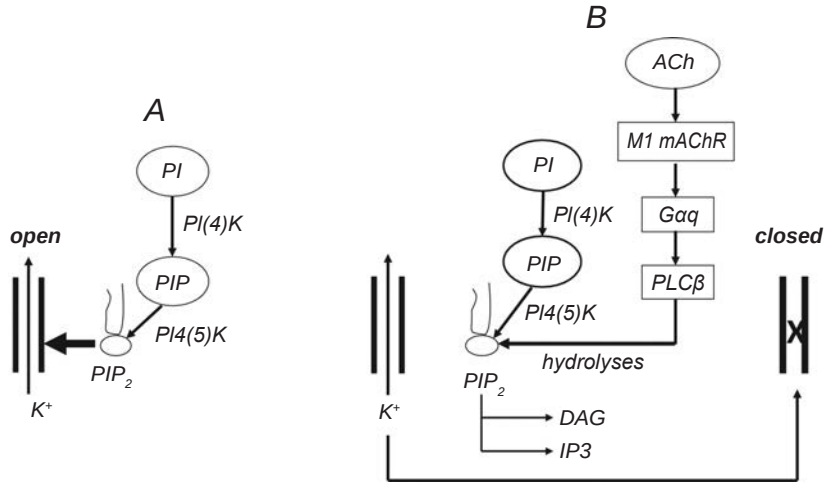
There are five subtypes of mAChRs, M1 through M5 [8]. They are all G protein-coupled receptors. M1, M3, and M5 couple preferentially to G proteins of the Gq family, Gq and G11; M2 and M4 receptors primarily activate Gi and Go. Most neurons in the rat superior cervical ganglion, SCG (the most frequently used experimental test object, as in Fig. 1) transcribe at least three of these, M1, M2, and M4, and a few also transcribe M3 [7].

M1 RECEPTORS

These are the receptors responsible for the effects shown in Fig. 1 [9, 10] and for the analogous effects in mice [11]. These effects are caused primarily through the closure of a set of voltage-gated K^+ channels originally termed M channels [12] but now known to be composed of subunits of the Kv7 K^+ channel family. In the rat SCG, these channels are (presumed) tetramers of Kv7.2 and Kv7.3 subunits [13, 14]. The above channels normally open when the neuron is depolarized or when it fires action potentials (APs); this induces strong adaptation of firing, severely limiting the frequency response of the neuron – a sort of excitability "brake" [15]. Closure of the M channels depolarizes the neuron (because a few channels are open at rest), strongly enhances firing in response to sustained or high-frequency depolarization, and may induce spontaneous firing (as in Fig. 1). The latter is probably explicable by a reduced threshold for the Na^+ current (as in hippocampal neurons [16]) coupled with depolarization and loss of adaptation.

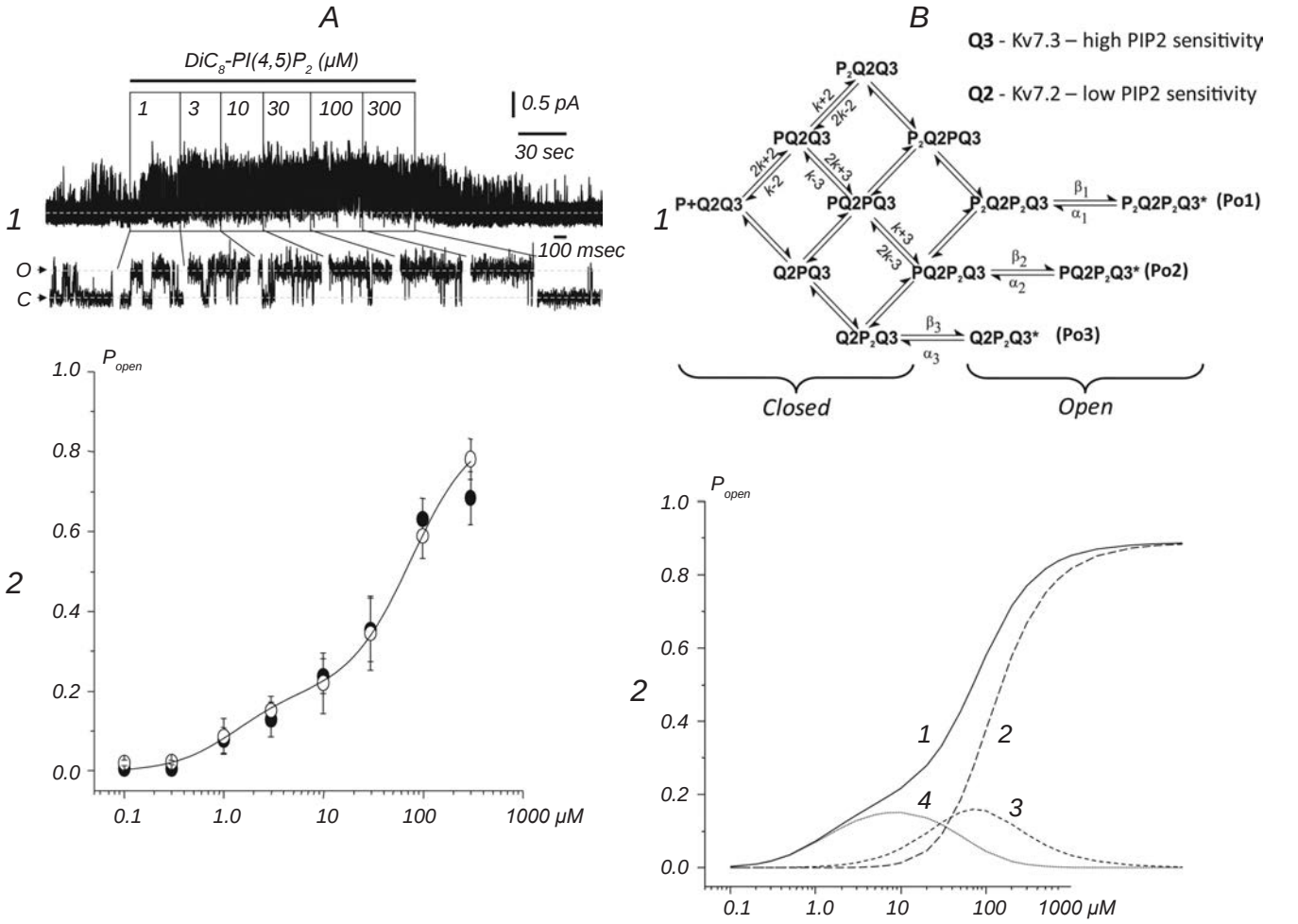
The biochemical pathway from the mAChRs to the channel has been reviewed by Delmas and Brown [17] and is summarized in Fig. 3. The key factor is that, though "gated" by voltage, the channels have an absolute requirement for the presence of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) in order to enter into; due to this, they can stay in the open state [18, 19]. M1 mAChRs couple to Gq and consequently activate phospholipase C(β) (PLCβ); membrane PIP2 is thereby hydrolysed and reduced to a level that cannot sustain channel opening. This depletion can be both profound (>90%) and rapid [20, 21], with a time course approaching that for M-current inhibition in SCG neurons [5].

The dependence on PIP2 means that the M channels can also be viewed as ligand-gated ion channels, with PIP2 as the ligand. Then, if the relation between the PIP2 concentration and channel opening is known, the effect of mAChR stimulation can be quantitated in terms of reduction in the PIP2 concentration [22]. However, the channel response to PIP2 is complicated by the fact that the two Kv7 subunits (Kv7.2 and Kv7.3) are characterized by a 100-fold difference in their sensitivities to PIP2 [19]. Thus, single Kv7.2/7.3 channels show a biphasic response to increasing concentrations of PIP2 (Fig. 4A). Using a standard kinetic scheme for multi-subunit channels, this was interpreted to indicate that, while all four subunits have to bind PIP2 for maximal channel opening, lower-frequency openings occur when only the two higher-sensitivity Kv7.3 channels bind PIP2 (B; see [23]). Physiologically, this subunit structure has the advantage that M channels are responsive over a wide



F i g. 3. Mechanism of cholinergic inhibition of M currents in a rat sympathetic neuron. A) Channels maintained in the open state by attachment of PIP2. B) Channels close because activation of M1 mAChRs leads to hydrolysis and loss of PIP2. Abbreviations: PI is phosphatidylinositol, PIP is phosphatidylinositol-4-phosphate, PIP_2 is phosphatidylinositol-4,5-bisphosphate, PI(4)K is phosphatidylinositol-4-kinase, PI4(5)K is phosphatidylinositol-4-phosphate-5-kinase; Gαq is α-subunit of Gq, PLCβ is phospholipase Cβ; DAG is diacylglycerol, and IP3 is inositol-1,4,5-trisphosphate.

Р и с. 3. Механізм холінергічного гальмування М-струмів у симпатичному нейроні щура.



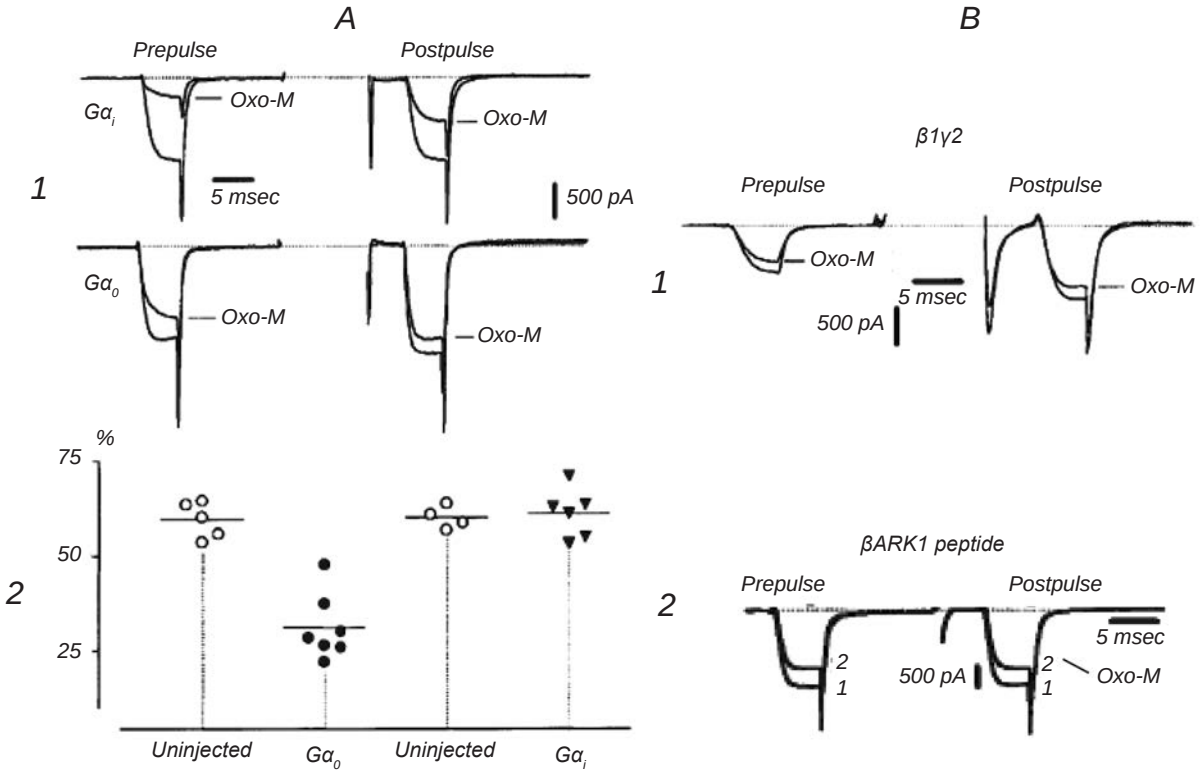
F i g. 4. Response of single Kv7.2/7.3 (M) channels to increasing concentrations of a water-soluble PIP2 analog, DiC_8 PIP2, and its mechanism. A1) Channel subunits had been co-expressed from their cDNAs in CHO cells. Upper record shows responses of a channel in an excised inside-out membrane patch to increasing concentrations of a PIP2 analog applied to the inside face; holding potential is 0 mV. A2) The graph shows the averaged responses (single channel open probability, P_{open}) from 5 to 14 such patches (open circles and line) and from 9 to 21 patches expressing concatenated Kv7. 2-7.3 subunits (filled circles); abscissa) concentration of DiC_8 - P(4,5) P.1, mM; ordinate) channel open probability. B1**)** Kinetic scheme for PIP2 activation of tetrameric Kv7.2/7.3 channels. Q2 is Kv7.2, Q3 is Kv7.3, P is PIP2, and Po is the open probability, P_{open}. The channel can open to low Po when the two Kv7.3 subunits bind PIP2 (Po2, Po3), but full opening requires all four subunits bound (Po1). Openings when the 2 Kv7.2 subunits bind PIP2 with none or only one of the Kv7.3 bind PIP2 are too rare to contribute to overall P_{open}. B2) The cumulative open probabilities accurately recapitulate the experimental data in A. 1) Model P_{open}, 2-4) Po1, Po2, and Po3, respectively. Adapted from [23], q.v. for details.

Р и с. 4. Відповіді поодиноких М-каналів Kv7.2/7.3 на прикладання водорозчинного аналога PIP2 – DiC_8 PIP2 – у зростаючих концентраціях.

range of PIP2 concentrations and, hence, less subject to small fluctuations in the PIP2 concentration while retaining sensitivity to the action of PIP2-depleting ACh.

M4 RECEPTORS

The principal effect of stimulating these receptors on sympathetic neurons is to inhibit the N-type (Ca_V2) voltage-gated Ca^{2+} channels [10]. In most part, this is mediated in a relatively direct manner; the M2 mAChR activates the G protein Go, and the βγ-subunits of Go interact directly with the Ca^{2+} channel to reduce its opening probability (see [24] and references therein). Thus, inhibition is largely prevented by specific antibodies or antisense RNA to Go (but not to Gi or Gq), is replicated by over-expressing free βγ-subunits, and is blocked by a βγ-sequestering peptide (Fig. 5). This form of Ca^{2+}-current inhibition is the somatic homolog



F i g. 5. M4 mAChR inhibition of I_{CaV2} in rat SCG neurons is mediated by βγ-subunits of the G protein Go. Records show Ca^{2+} currents generated by 5-msec-long steps from –70 mV to +5 mV before (prepulse) and after (postpulse) a 10-msec-long step to +90 mV (this temporarily reverses G protein-induced suppression because the latter is voltage-dependent). Twenty mM BAPTA in patch pipettes. A1) Records from neurons preinjected with antibodies to Gi and Go α-subunits. The muscarinic agonist oxotremorine-M (Oxo-M, 10 μM) inhibits I_{Ca} with Gi-Ab, and inhibition is reversed by depolarization to +90 mV. Go-Ab selectively reduces inhibition. A2) The respective graph; vertical scale) normalized intensity of inhibition of I_{Ca}, %. B1) Records show that expression of β1γ2 G protein β-subunits from their cDNAs inhibits I_{Ca} and occludes inhibition by Oxo-M. Lower records (2) show that expression of the βγ-sequestering peptide, βARK1, from its cDNA also prevents inhibition by Oxo-M. Adapted from [24].

Р и с. 5. Гальмування струмів I_{CaV2}, опосередковане мускариновими АХ-рецепторами підтипу М4, у нейронах верхнього шийного ганглія щура (ефект опосередковується βγ-субодиницями G-протеїну Go).

of the process responsible for muscarinic inhibition of noradrenaline release from postsynaptic sympathetic nerve endings [25]. However, such inhibition is excitatory, rather than inhibitory, in the soma, since the reduced Ca^{2+} influx during the AP reduces activation of the small-conductance SK3 Ca^{2+}-dependent K^+ channels that generate after-hyperpolarization. Reduction of the SK3 current reinforces the effect of M current inhibition in enhancing the frequency of AP discharges [26]).

Interestingly, like the M channels, these N-type Ca^{2+} channels are also regulated by PIP2 [27] and hence are also inhibited by activating M1 mAChRs [10], although rather more slowly than by the M4 mAChRs. This PIP2-dependent inhibition does not seem to be present at the sympathetic nerve endings [25] but is very substantial in the somata and undoubtedly contributes greatly to the reduction of SK current. Thus, over-expression of a constitutively active Gq α-subunit completely suppresses both M current and SK current [28] and produces a most profound increase in the frequency and duration of depolarization-induced spike discharges in the rat SCG [29].

M2 RECEPTORS

In cell lines used as expression systems [30], M2 mAChRs can inhibit N-type Ca^{2+} currents just as well as M4 receptors. Indeed, in some neurons (including

mouse sympathetic neurons [31]) it is the endogenous M2 receptor that is responsible for Ca_V2 channel inhibition, not the M4 receptor [32]. However, in spite of the expression of both M2 and M4 mRNAs in rat sympathetic neurons, it is only the M4 mAChRs that drive Go-mediated Ca^{2+} current inhibition [10, 24]. Instead, the main effect of stimulating M2 mAChRs is to activate the inward rectifier Kir3 current. This current is very small in most rat SCG neurons but can be enhanced by cDNA transfection. Stimulation of the M2 mAChRs then strongly increases this current through preferential activation of the G protein Gi and thence by a direct effect of the associated βγ-sububits in the Kir channels [33, 34]. This is presumably responsible for cholinergic slow inhibitory postsynaptic potentials seen more clearly in some frog sympathetic neurons [35].

Interestingly and conversely to Ca_V2 inhibition, the endogenous M4 mAChRs do not activate the Kir3 channels [33]. Thus, in summary, we have the interesting dichotomy for M2 and M4 mAChR signaling in the rat SCG neuron.

SEGREGATION OF MUSCARINIC RECEPTOR SIGNALLING PATHWAYS

Since M2 and M4 mAChRs in open reconstituted systems can couple with a near-equal avidity to both Gi and Go G proteins and can affect both Kir3 and Ca_V2 channels, there must be some mechanism for microanatomical segregation of the two receptors and their cognate signalling partners in the sympathetic neuron. This apparent segregation can be broken by, e.g., inactivation of endogenous Gi to prevent M2 mAChR from activating Kir3 and subsequent over-expression of Go. M2 mAChRs then activate Kir3 using Go instead of Gi [34]. There is also strong evidence for segregation of the GPCR receptor Ca^{2+}-signalling systems in sympathetic neurons. Thus, M1 mAChR stimulation does not normally cause a rise in the intracellular [Ca^{2+}] in such neurons (as might have been expected from hydrolysis of PIP2 to IP3, since this usually releases Ca^{2+} from the endoplasmic reticulum, ER). At the same time, stimulation of the Gq-coupled bradykinin B2 receptors (B2Rs) does release Ca^{2+} [36]. This is because the B2R is closely coupled to the IP3 receptor on the ER, whereas the M1 mAChR is not [37]. In fact, the latter is "protected" from releasing Ca^{2+} by calmodulin [37] and by the IP3R-binding protein IRBIT [38]. However, as with the M2/M4 segregation, this restraint on M1 mAChR Ca^{2+} signalling can be broken by over-expression of the M1 mAChRs [38], while the B2R-IP3R microdomain can be broken by disrupting the cytoskeleton [32]. Finally, there is a converse microdomain linking the M1 mAChR directly with the M channel via the A-kinase-anchoring protein AKAP79/150 that is not shared by the B2R [39,40,41]. AKAP79/150 acts as a scaffold to facilitate phosphorylation of the M channel subunits by protein kinase C when it is activated by diacylglycerol formed from PIP2 hydrolysis (Fig. 3B). This in turn enhances M1 mAChR-induced channel inhibition [39, 41], probably by reducing the sensitivity of the channels to PIP2 [17, 42] and so increasing their closure reaction when PIP2 is hydrolysed.

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ІНШІ (МУСКАРИНОВІ) АЦЕТИЛХОЛІНОВІ РЕЦЕПТОРИ В СИМПАТИЧНИХ ГАНГЛІЯХ: ЕФЕКТИ АКТИВАЦІЇ ТА МЕХАНІЗМИ

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Р е з ю м е

Ацетилхолін, котрий вивільнюється з прегангліонарних волокон, може активувати в симпатичних гангліях ацетилхолінові рецептори двох типів – нікотинові та мускаринові. Перші з них мають ліганд керовані іонні канали, відповідальні за пряму синаптичну передачу; другі ж є рецепторами, зв'язаними з G-протеїнами, та опосередковують різні непрямі модуляторні ефекти. У більшості симпатичних нейронів ссавців експресуються мускаринові рецептори трьох підтипів – M1, M2 та M4; у деяких також експресуються рецептори підтипу M3. Активація рецепторів M1 зумовлює стимуляцію G-протеїну Gq; це викликає повільну деполяризацію постсинаптичного нейрона та підвищення його збудливості, що, кінець кінцем, призводить до генерації асинхронного розряду потенціалів дії. Така генерація може проривати „нікотинове" блокування ганглія. Цей ефект в основному опосередковується закриванням потенціалкерованих калієвих М-каналів, що складаються із субодиниць Kv7.2 та Kv7.3, завдяки гідролізу та вичерпанню запасів мембранного фосфатидилінозитол-4,5-бісфосфату. Активація рецепторів М2 призводить до гіперполяризації

та гальмування постсинаптичного нейрона в результаті відкривання G-протеїнкерованих калієвих каналів внутрішнього випрямлення Kir, опосередкованого G-протеїном Gi. Рецептори M4 гальмують кальцієві канали N-типу CaV(2); ефект опосередковується G-протеїном Go. У сомах постгангліонарних нейронів це зумовлює збільшення збудливості (через зменшення кальційзалежних калієвих струмів). У відростках постгангліонарних нейронів та аксонних терміналях, навпаки, CaV(2)-опосередковане гальмуванням зменшує вивільнення норепінефрину та пригнічує постгангліонарну передачу. Різні мускаринові рецептори можуть бути анатомічно відокремленими одні від інших завдяки локалізації їх споріднених G-протеїнів та (у деяких випадках) іонних каналів у різних сигнальних мікродоменах.

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