Br. J. Pharmac. (1983), **80**, 235–240

Analysis of current noise evoked by nicotinic agonists in rat submandibular ganglion neurones

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1 The kinetics of the responses of rat submandibular ganglion neurones to nicotinic agonists were investigated by the analysis of agonist-induced membrane current noise.

2 Two kinetic components are found in the responses of these cells to acetylcholine, carbachol, suberyldicholine, dimethyl-4-phenylpiperazinium and nicotine, in agreement with previous findings of two components in evoked synaptic currents in these cells.

3 Small, though significant, differences in the relative amplitudes of the two kinetic components were observed in the spectra of noise evoked by the different agonists. In particular, the fast component in the response to carbachol was relatively larger with respect to the slow component than with any other agonist tested.

4 No measurable dependence of the relative sizes of the two components on agonist concentration or membrane potential was observed.

5 The evidence supports the hypothesis that the two kinetic components are the product of two independent channel populations for which the agonists tested show some slight differences in selectivity.

Introduction

Acetylcholine, and other nicotinic agonists, evoke in rat submandibular ganglion cells an increase in cation conductance that leads to an inward depolarizing current. The same receptors and ion channels are responsible for the fast excitatory synaptic current in these cells (Ascher, Large & Rang, 1979; Rang, 1981). In many respects this response closely resembles the response of the motor endplate (see Peper, Bradley & Dreyer, 1982 for review) or of *Aplysia* neurones (Ascher, Marty & Neild, 1978a) to nicotinic agonists. However, one important difference is that the ganglion cell response to either applied agonists or to the synaptic release of acetylcholine, consists of two distinct kinetic components (Rang, 1981), one of which has an unusually long mean channel lifetime (about 50 ms at −80 mV membrane potential and 20°C). The other (fast) component has a mean channel lifetime close to 9 ms at this potential. This has not generally been observed in other autonomic ganglion cells where only one population of channels has usually been found (Kuba & Nishi, 1979; MacDermott, Connor, Dionne & Parsons, 1980). Various nicotinic antagonists have been found to show some degree of selectivity between the fast and the slow components of the response (Rang, 1982). In particular tubocurarine shows strong selectivity in blocking the slow component, whereas hexamethonium more strongly affects the fast component. In this study we have investigated the selectivity of agonists in activating these two components, using the analysis of membrane current noise (Katz & Miledi, 1972; Anderson & Stevens, 1973) to distinguish the contributions of the two types of receptor to the overall membrane response. A noise spectrum consisting of two components can be represented by the equation:

$$G(f) = \frac{G_1(0)}{1 + (f/f_{1C})^2} + \frac{G_2(0)}{1 + (f/f_{2C})^2}$$

Where G(f) is the one-sided spectral density
$G_1(0)$ and $G_2(0)$ are the zero frequency asymptotes of the fast and slow components respectively;
f_{1C} and f_{2C} are the two half power (cut-off) frequencies of the two components.

At low concentrations, where the channel spends only a small proportion of the time in the open state (see Katz & Miledi, 1972; Anderson & Stevens, 1973), the noise variance is directly proportional to the mean current, I

$$\text{var}(I) = I \cdot i$$

where i is the current flowing through a single channel.

If, as argued by Rang (1981) the two components represent two populations of channels with different kinetic characteristics, at low concentrations the total current flowing through each type of channel is proportional to the variance var(I) of the current associated with each component, which can be calculated from the parameters of the calculated spectrum

$$\text{var}_1(I) = (\pi/2) \cdot G_1(0) f_{1C}$$
$$\text{var}_2(I) = (\pi/2) \cdot G_2(0) f_{2C}$$

For the two component spectrum, therefore

$$f_{var} = \frac{\text{var}(I_1)}{\text{var}(I_1) + \text{var}(I_2)} = \frac{I_1}{I_1 + (i_2/i_1)I_2}$$

The left hand side then expresses the variance of one component (the fast, say) as a fraction of the total variance. If the ratio I_1/I_2 or i_1/i_2 varies between different agonists, then f_{var} will also vary. A difference in I_1/I_2 for different agonists would suggest that they may, like some antagonists, differ in their relative potency on the two types of receptor; a difference in i_1/i_2 would imply that the conductance of one or both types of channel can vary according to the agonist.

The results show that, among the five agonists tested there are only small differences in selectivity, though the mean open time, particularly of the slow channels varies appreciably between different agonists.

Methods

The methods used were essentially the same as those described by Rang (1981). Submandibular ganglia from male Listar rats of 150–200 g were pinned in a chamber lined with Sylgard resin: the ganglia were cleaned as far as possible by manual removal of the connective tissue covering the neurone somata. Experiments were carried out on the stage of a microscope fitted with Nomarski optics and a 40 × water immersion objective. The bath was perfused with Krebs solution (composition mM: NaCl 119, KCl 4.7, $CaCl_2$ 5, KH_2PO_4 1.2; $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 11) equilibrated with 95% O_2: 5% CO_2; the increased calcium concentration was found to improve cell survival following electrode penetration. Drugs were either added to the perfusing solution or applied by ionophoresis. When acetylcholine was used 2.5 μM neostigmine was added to the bathing solution. The bath temperature was maintained at 20° ± 1°C (as measured by a bath mounted thermistor) by controlling the temperature of the inflow solution.

High resistance electrodes had to be used for successful impalements: voltage electrodes were about 50 MΩ (filled with KAc 4 M) and current electrodes about 80 MΩ (filled with K_2SO_4 0.5 M). This limited the settling time and high frequency response of the clamp. However, the clamp circuit used by Rang (1981) was modified to allow negative capacity compensation of the current electrode, which maintained the linearity of the current-voltage converter to higher frequencies, and independent control of high and low frequency feedback gain of the clamp amplifier using a method similar to that described by Smith, Barker, Smith & Colburn (1980). These changes produced some improvement at the high frequency end of the spectra.

The current signal was sampled for 60–120 s at a sampling frequency of 256 Hz via a bandpass filter set at 0.2 and 120 Hz. Net power spectra, obtained from the difference of spectra recorded in the presence and absence of agonist, were calculated at 0.5 Hz resolution and fitted from 0.5 to 49.5 Hz using a least squares fitting routine on a PDP 11 computer (see Colquhoun, Dreyer & Sheridan, 1979). The routine calculated estimates of the parameters of the spectra and the standard deviations (s.d.) of these estimated parameters (see Colquhoun, Rang & Ritchie, 1974); in the few cases where the s.d.s exceeded ±50% of the parameter values, spectra were rejected. It was found impossible to hold cells and drug-induced currents at potentials much depolarized or hyperpolarized from the resting potential for the long periods needed for good spectra. Hence most recordings were made at −60 mV or −80 mV, with a few at −40 mV.

Results

As was shown by Rang (1981) noise spectra recorded in the presence of nicotinic agonists could not be fitted by a single Lorentzian component but were well fitted up to 50 Hz by the sum of two Lorentzians (Figure 1a,c). Spectra recorded in the presence of various agonists show differences in the relative size of the two components, expressed as f_{var} (Table 1) (Figures 1 and 2). The relative size of the fast component was greater in the presence of carbachol (CCh) than in the presence of acetylcholine (ACh), dimethyl-4-phenylpiperazinium (DMPP), suberyldicholine (SubCh) or nicotine (Nic). Within the narrow range of concentration (CCh 25 μM and 50 μM) and membrane potential (−60 mV to −80 mV) covered, no significant effects of these variables upon f_{var} were observed. The ratio of the estimate of f_{var} in the presence of 25 μM CCh to that in the presence of 50 μM CCh was 1.03 ± 15% (ratio of the means ± coefficient of variance), the mean ratio for ACh, CCh and DMPP at −60 mV and −80 mV was 1.06 ± 13%.

Time constants calculated from the half power

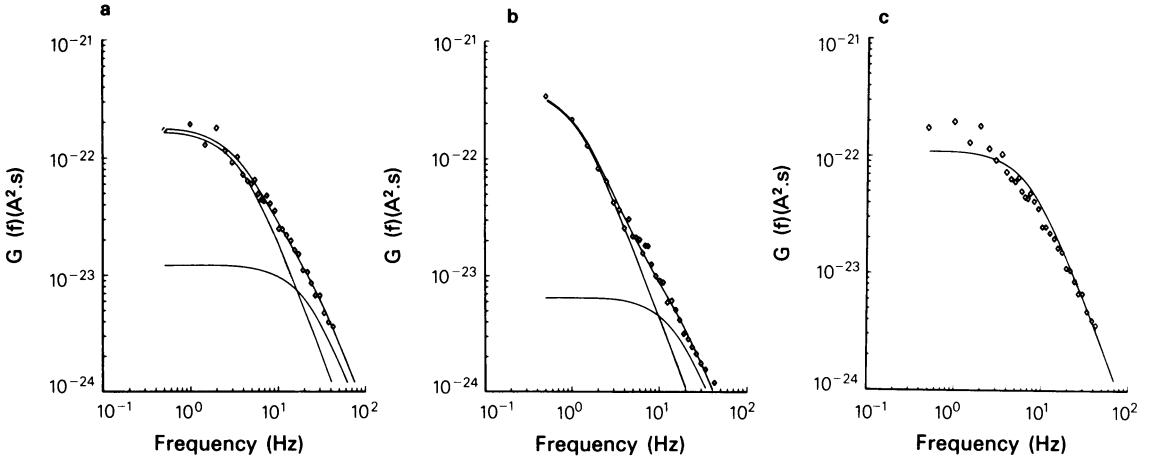

Figure 1 Power spectra from a single cell at −60 mV holding potential of carbachol (CCh) and suberyldicholine (SubCh)-evoked current fluctuations: (a) and (c) 50 μM CCh; (b) 5 μM SubCh. The spectra in (a) and (b) have been fitted with the sum of two Lorentzian components; the plotted lines are the fitted curve and the two Lorentzians plotted individually, (c) shows the same spectra as (a) fitted with a single Lorentzian component. The half power frequencies of the fitted curves are (a) 3.3 Hz and 20.5 Hz; (b) 1.1 Hz and 15.0 Hz; (c) 7.2 Hz.

frequencies of the two components in the presence of various agonists are given in Table 1. If the channel is taken as having a single open state and any number of rapidly equilibrating closed states then it can be shown that such behaviour generates a single Lorentzian noise spectrum with time constant

$$\tau = 1/2\pi f_c$$
$$= p_T(\infty)/\alpha$$

where f_c is the half power frequency and $p_T(\infty)$ the equilibrium proportion of closed channels (see for example, Colquhoun & Hawkes, 1977). A commonly used example of such a model is the KM model (e.g. Castillo & Katz, 1957; Katz & Miledi, 1972):

$$(1) \qquad A + T \underset{k-1}{\overset{k+1}{\rightleftharpoons}} AT \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} AR$$
$$\text{closed} \qquad \text{open}$$

Binding is assumed to be fast in relation to the conformation change leading to channel opening (Anderson & Stevens, 1973). Thus if the two compo-

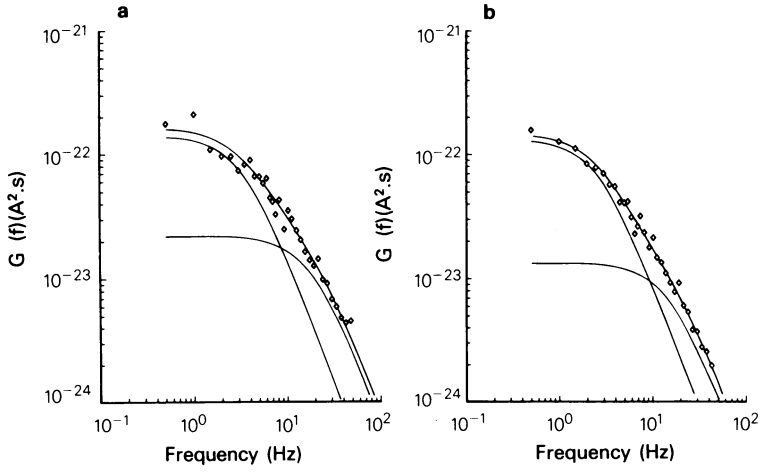

Figure 2 Power spectra from a single cell at −60 mV holding potential of carbachol (CCh) and dimethyl-4-phenylpiperazinium (DMPP)-evoked current fluctuations: (a) CCh 50 μM; (b) DMPP 5 μM. Both spectra have been fitted with the sum of two Lorentzian components; the plotted lines are as in Figure 1. The half power frequencies of the fitted curves are (a) 3.2 Hz and 17.7 Hz; (b) 2.6 Hz and 14.8 Hz.

Table 1 Values from noise analysis of f_{var}, τ_2, τ_1 and γ at −60 mV (±s.e.mean)

Agonist	f_{var}	τ_2(ms)	τ_1(ms)	γ(pS)	*n*
CCh	0.34* (±0.02)	42.4 (±2.9)	6.2 (±0.5)	18.6 (±1.1)	37
DMPP	0.24 (±0.02)	51.0 (±3.6)	6.9 (±0.6)	19.7 (±1.2)	21
SubCh	0.20 (±0.02)	85.6 (±5.1)	7.6 (±0.5)	17.3 (±1.2)	12
ACh	0.21 (±0.03)	79.2 (±9.4)	6.5 (±1.3)	19.7 (±2.2)	11
Nic	0.23 (±0.03)	56.0 (±6.7)	6.2 (±0.6)	21.4 (±2.4)	12

CCh = carbachol; DMPP = dimethyl-4-phenylpiperazinium; SubCh = suberyldicholine; ACh = acetylcholine; Nic = nicotine.

Differences between the f_{var} value for CCh and the f_{var} values for all the other agonists are significant at least the 1% level.

Those between the other agonists are not significant.

nents in the spectra are supposed to originate from two independent channel populations, τ_1 and τ_2 can be interpreted as $1/\alpha_1$ and $1/\alpha_2$ (the mean channel lifetimes) respectively, provided that $p_T(\infty)$ is close to 1. The drug-induced mean currents were between 0.5 and 1.5 nA at −60 mV, whereas evoked synaptic currents (e.s.c.s.) are about 15 nA at the same potential. Hence the probability of a channel being open was not more than 0.1 and $p_T(\infty)$ not less than 0.9. On these assumptions, lifetimes range from 6.2 ms (CCh) to 7.6 ms (SubCh) for the fast component and from 42.4 ms (CCh) to 85.6 ms (SubCh) for the slow component at −60 mV. At the frog neuromuscular junction the mean lifetime of the channels opened by SubCh exceeds that of channels opened by CCh by a factor 4.1–5.5 (Katz & Miledi, 1972; 1973; Neher & Sakmann, 1975). The difference between these two agonists is present, but much smaller in the ganglion cells, the ratio SubCh/CCh being 2.0 for the slow channels and 1.2 for the fast channels.

The concentration-dependence of τ_s and τ_f was studied over a twofold range for two agonists, τ(25 μM)/τ(50 μM) for CCh was 1.14 ± 20% (ratio of the means ± coefficient of variance) for the slow component and 0.97 ± 20% for the fast component. With DMPP τ(2.5 μM)/τ(5 μM) was 1.11 ± 11% for the slow component, 0.97 ± 24% for the fast. None of these effects was statistically significant.

The voltage-dependence of τ_1 and τ_2 shows no clear relationship to the agonist used. The pooled value of $\tau_2(-80)/\tau_2(-60)$ for ACh, CCh, DMPP and Sub was 1.21 ± 0.05 (mean ± s.e.mean; $n = 33$) and of $\tau_1(-80)/\tau_1(-60)$ was 1.19 ± 0.11 (mean ± s.e.mean; $n = 33$). Expressed as the potential change (H) for an e-fold change in τ these become H = 105 mV for the slow component, and H = 115 mV for the fast component, values which are close to the voltage-dependence of the e.s.c.s in the same preparation (Rang, 1981) and of the endplate currents in frog muscle (Magleby & Stevens, 1972).

The well known effect of nicotine in causing initial ganglion depolarization followed by repolarization and synaptic block (see Paton & Perry, 1953), suggested that it might possess channel blocking as well as agonist activity, as has been shown for decamethonium at the endplate (Adams & Sakmann, 1978b). If this were occurring it would be expected to distort appreciably the voltage-dependence of the time constants and areas of the two components of the noise spectra (see discussion). We have examined the action of nicotine over the voltage range −40 to −80 mV. In 6 cells, the value of $\tau(-80)/\tau(-40\text{ mV})$(±s.e.mean) was 1.38(±0.28) for the slow component and 1.11(±0.22) for the fast component. These are not significantly different from the pooled values quoted above when corrected for the difference in potential step. The relative contribution of the fast component to the total variance, expressed as f_{var}, appeared to be rather greater at −40 mV than at −80 mV, the ratio $f_{var}(-40\text{ mV})/f_{var}(-80\text{ mV})$ being 1.8 ± 0.32 (6 cells). This difference is not statistically significant at the 5% level, but suggests that the fast component may tend to become smaller relative to the slow component as the cell is hyperpolarized. This could arise if nicotine had some channel blocking activity in respect to the fast channels. Since it is known to act as a cation (Barlow, 1965) hyperpolarization would enhance the channel block, producing a relative reduction of the current through the fast channels. We tested whether appreciable channel block occurred with nicotine by measuring the steady state current-voltage relationship for the nicotine-induced inward current, comparing it with the curve for carbachol, which shows no appreciable channel blocking activity (Ascher *et al.*, 1979), as described by Adams & Sakmann (1978a). Figure 3 shows the normalised

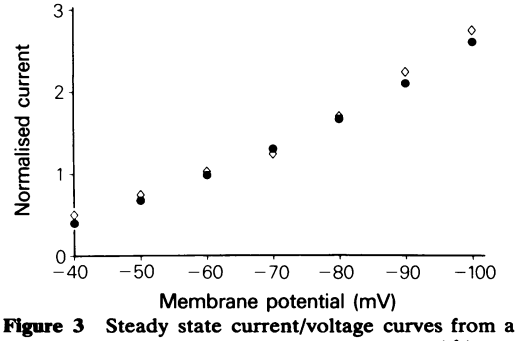

Figure 3 Steady state current/voltage curves from a single cell in the presence of 12.5 μM carbachol (◇) and 2.5 μM nicotine (●). The current has been normalised to 1 at −60 mV.

current–voltage curves from a cell perfused with 12.5 μM CCh and with 2.5 μM nicotine. The curves are virtually superimposable, and there is no sign of the relative reduction in steady state current with nicotine compared to CCh at hyperpolarized potentials which would be expected if it had appreciable channel blocking activity.

The unit conductance (γ) was calculated from the expression:

$$\gamma = \frac{\pi(G_1(0) \cdot f_{1c} + G_2(0) \cdot f_{2c})}{2I(V - V_{eq})}$$

Where $G_1(0)$ and $G_2(0)$ are the zero frequency spectral densities for the fast and slow components, I the mean agonist induced current, V the membrane potential and V_{eq} the equilibrium potential. V_{eq} was taken as −10 mV (Rang, 1981). This equation gives a weighted mean value of γ for the two types of channel, and assumes that both channel types have the same conductance. Calculated values are given in Table 1 and show no significant agonist-dependence, ranging from 17.3 pS (SubCh) to 21.4 pS (Nic). These values are somewhat smaller than those found at the frog endplate (for review, see Colquhoun, 1979). It is notable that the calculated value for CCh (for which the fast component is relatively larger than for other agonists) agrees with the value for the other agonists. If γ were markedly different for the slow and fast channels, CCh would be expected to give a discrepant value. It is therefore likely, as concluded by Rang (1981) that the conductances of the slow and fast channels are similar.

Discussion

The main conclusions of this paper are that agonists can vary in respect of the proportion of agonist-induced current that flows through the 'slow' and 'fast' channels; and that mean channel lifetime for both slow and fast channels varies according to the agonist used. Before accepting the first conclusion it is necessary to be sure that it does not represent an artefact of the fitting procedure. This is made unlikely by the fact that the estimated half power frequencies are similar for DMPP and CCh, though f_{var} is different, whereas DMPP and SubCh have different half power frequencies but similar values of f_{var}. The fact that there is no evident correlation between the two makes it unlikely that the variation of f_{var} is artefactual.

If the presence of two components is attributed to two independent populations of channels then the value of f_{var} measures the relative current carried by the two types of channel (provided that the proportion of open channels is small; see results). But if the presence of two components in the spectra is taken as representing the kinetics of a multistate receptor/channel interaction (for example it might represent the presence of bound but closed as well as open states, Dionne, 1981; or channel block by the agonist, Adams & Sakmann, 1978b), then changes in f_{var} imply changes in the relative values of the rate constants which describe transitions between the various states. As some of these rate constants are voltage- or concentration-dependent the value of f_{var} in such models, will in general be expected to show concentration- or voltage-dependence (as would the half power frequencies of the components) (see Adams, 1977; Ascher, Marty & Neild, 1978b; Ascher *et al.*, 1979; Dionne, 1981). No such effects on either f_{var} or the half power frequencies were observed here for any of the agonists, including nicotine. Although the range of concentration and membrane potential examined was small, the results are in agreement with the lack of effect of changing ACh concentration on the time constants or relative amplitude of the two components of voltage jump relaxations (Rang, 1981), and support his conclusion that the two components represent two independent channel populations.

One interpretation of the agonist-dependence of f_{var} if there are two independent channel populations, is that the relative conductance of the fast and slow components varies for different agonists. In this case some effect upon mean conductance is likely (though not inevitable). No significant effect was seen, though a small effect could have passed unnoticed. However, such an explanation is unlikely as agonist-dependence of channel conductance has not generally been observed elsewhere (Colquhoun, 1979).

The observed variation of f_{var} can therefore best be interpreted by supposing that the two channel types have different probabilities of being open in the presence of the same concentration of different agonists. These changes are partly explained by changes in channel lifetimes but not entirely. For the model in eqn. 1 the probability of the channel being in the open state P_{open} is, for low agonist concentrations:

$$P_{open} \simeq (\beta/\alpha) \cdot (x_A/K)$$

where x_A is the agonist concentration and K the dissociation constant for agonist binding. The value of f_{var} can be expressed as $P_1/(P_1 + P_2)$ where P_1 and P_2 are the probabilities of opening for the channel populations 1 and 2 respectively.

$$\text{Thus } f_{var} = P_1/(P_1 + P_2) = \frac{(\beta_1/\alpha_1 \cdot x_A/K_2)}{(\beta_1/\alpha_1 \cdot x_A/K_1 + \beta_2/\alpha_2 \cdot x_A/K_2)}$$

Thus if the changes in f_{var} depend solely on changes in relative lifetime then the value of f_{var} would be proportional to $1/\alpha_1/(1/\alpha_1 + 1/\alpha_2)$., i.e. $\tau_1/(\tau_1 + \tau_2)$ at low values of P_{open}. Making this allowance for

changes in relative channel lifetimes the expected relative area for DMPP becomes 0.32, still significantly different ($P < 0.05$) from the measured value (0.24). Thus changes other than the relative channel lifetime must also be involved, possibly the relative affinity K_1/K_2 of the agonist for the receptors controlling the two types of channel. In this connection it is interesting that trimetaphan, an antagonist which probably acts by blocking receptors (Ascher *et al.*, 1979) appears to have a higher affinity for slow rather than fast receptors (Rang, 1982).

We conclude that agonists show a measurable degree of selectivity in opening slow and fast channels respectively. The selectivity is small, however, and our hope of finding agonists that discriminate unequivocally between the two kinds of channel has not been fulfilled.

We are grateful to Dr D. Colquhoun for helpful discussions. This work was supported by the M.R.C.

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(Received February 24, 1983. Revised May 18, 1983.)