Catechol and Noradrenaline Facilitate the Neuromuscular Transmission *via* Different Mechanisms

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Summary Using curarized frog sartorius muscles, selective presynaptic facilitatory effects of two structurally related chemicals, noradrenaline (NA) and catechol (pyrocatechol, PC), were compared by calculating the fractional release (P) from the rundown of endplate potential during a train-of-three stimulation. Below 10 μM , both NA and PC increased P in a similar manner. At higher concentrations, however, the effects of NA were found to be quite different from those of PC. NA never increased further the value of P (0.11–0.13) whereas PC augmented P in a dose dependent fashion up to about 0.76. Thus, the present data suggested that NA and PC facilitate the transmitter release *via* different mechanisms. The effects of the latter were identical to the case of “chemical potentiation or accelerated evoked release” by 4-aminopyridine.

Key Words: catechol, noradrenaline, transmitter release.

Hydroxylated benzenes such as catechol (pyrocatechol, PC) and phenol are known to possess a profound facilitatory effect on neuromuscular transmission (COPPÉE, 1943; OTSUKA and NONOMURA, 1963; BLABER and GALLAGHER, 1971; GALLAGHER and BLABER, 1973). The site of action of PC seems to be the presynaptic terminal, since it increases both the quantal content of endplate potentials (EPPs) and the frequency of miniature endplate potentials (mEPPs) without altering the amplitude of the mEPPs (GALLAGHER and BLABER, 1973). Similarly, micromolar doses of noradrenaline (NA) and other sympathomimetic amines that stimulate α -adrenoreceptors have been reported to potentiate the amplitude of the EPP and increase the frequency of mEPPs (KRNEVIĆ and MILEDI, 1958; JENKINSON *et al.*, 1968; KUBA, 1970; KUBA and TOMITA, 1971). In the case of NA, the target of action was ascribed to be the presynaptic terminal, since the postsynaptic parameters of neuromuscular transmission were not affected (JENKINSON *et al.*, 1968; KUBA, 1970).

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Table 1. Effects of micromolar doses of NA and PC on the fractional release (P).

Test drugs	Concentrations (μM)			
	1	2	5	10
Noradrenaline	—	1.03 ± 0.04	1.09 ± 0.07	1.21 ± 0.07
Catechol	1.12 ± 0.09	1.27 ± 0.07	1.69 ± 0.18	—

NA and PC data were obtained from the same muscle in the following sequence: control-NA-washout (2nd control)-PC. To normalize the data, all the figures were expressed in test/control ratio. The concentration of Ca was adjusted to 10 mM. Average $\pm$ standard deviation on 6 measurements.

PC seems to act on receptors other than α -adrenoreceptors (BLABER and GALLAGHER, 1971). However, since NA and PC have a related molecular structure and a common facilitatory drug action, it might be expected that they modify the same process in the transmitter releasing mechanism. Indeed, NA and PC were reported to stimulate the activities of Na^+ , K^+ -ATPase and Mg^{2+} -ATPase in the synaptosomes of rat cerebral cortex (SAWAS and GILBERT, 1981). To elucidate whether NA and PC, in fact, interact with the same process in the excitation-secretion coupling, their facilitatory actions were compared by calculating the fractional release (P) (MAENO *et al.*, 1981). The EPPs were recorded from curarized sartorius muscles of the frog, *Rana nigromaculata*. Three stimuli of 0.1–0.3 sec intervals were applied to the nerve fiber. P was computed from the following formula where P_t is the apparent fractional release, and V_0 and V_t are the amplitudes of the first and second EPP responses in a train, respectively:

$$P_t = (V_0 - V_t) / V_0.$$

P_t is an exponential function of the stimulus interval (t); namely $P_t = Pe^{-kt}$ (MAENO *et al.*, 1981). When t is small (*e. g.* 0.1–0.3 sec) e^{-kt} can be approximated to unity, and we obtain $P_t = P$. Consequently, we are able to estimate a fairly accurate value of P . It has already been shown by MAENO *et al.* (1981) that P serves as a useful index in analyzing the transmitter output. The amount of *d*-tubocurarine (dTC) in each test solution was adjusted so that the first EPP in a train was of almost the same amplitude in a series of experiment. Drugs used were *l*-noradrenaline bitartrate (Wako), pyrocatechol (Wako), and *d*-tubocurarine (Wako).

At micromolar doses (below 10 μM), NA and PC have been reported to increase the amplitude of EPP (OTSUKA and NONOMURA, 1963; JENKINSON *et al.*, 1968; KUBA, 1970; GALLAGHER and BLABER, 1973). In the present experiments, this was found to be associated with an acceleration of the rundown of EPPs (Table 1). The dose-release relation of the two drugs looked as if they were in parallel; PC had about a six times stronger effect than NA. However, at higher concentrations PC modified the transmitter release quite differently from NA (Fig. 1A). The effect of PC on P was more pronounced than that of NA which was small over a wide range of concentrations (10 μM to 1 mM).

Fig. 1. A: dose-release curves of NA (filled circles) and PC (open circles). Fitted curve for PC was calculated from the equation (MAENO *et al.*, 1981) with the following fixed parameters: $[\text{Ca}] = 10 \text{ mM}$, $K_A = 35 \mu\text{M}$, $K_{Ca} = 0.6 \text{ mM}$, $P_A = 0.76$, and $P_{Ca} = 0.11$. The data for PC were averages on 6 measurements, but the data for NA were from a single muscle. The vertical bar shows standard error of the mean. Dotted line gives the level of control P . B: modified Lineweaver-Burk plot of the NA data. Because of fourth power relation between the transmitter output and the external Ca concentration, we can expect a linear relation between $P^{-1/4}$ and $[\text{Ca}]^{-1}$. Open circles, control; filled circles, in $50 \mu\text{M}$ NA. P_{Ca} was estimated graphically from the intercept on Y-axis to be 0.11 for control and 0.14 for $50 \mu\text{M}$ NA. However, K_{Ca} calculated from the intercept on X-axis remained unaltered (0.57 mM). Averages of 7 and 5 measurements in control and $50 \mu\text{M}$ NA, respectively.

It should be pointed out that the pharmacological dose-release relationship of PC obtained in the present paper is identical in many respects to the case of "chemical potentiation or accelerated evoked release" (KATZ and MILEDI, 1979; MAENO *et al.*, 1981) of 4-aminopyridine (4AP). The amount of dTC required to suppress the EPP potentiated by 1 mM PC (55–75 μ M) was comparable to that of 1 mM 4AP (66–75 μ M). Such an enormous potentiation with PC was associated with a prolongation of the nerve terminal action potential (Maeno, unpublished) as seen with 4AP (ENOMOTO and MAENO, 1981). However, the two drugs did not alter the time course of extracellularly recorded endplate currents. The hypothetical equation proposed to explain the presynaptic action of 4AP (MAENO *et al.*, 1981) could also be applied in the present case of PC. Furthermore, the antagonistic interaction of streptomycin (SM) on the dose-release relation of PC (Maeno, unpublished) was the same as that observed with 4AP (MAENO *et al.*, 1981). However, peculiar multiple EPP responses seen in the presence of 4AP (LUNDH, 1978; MAENO *et al.*, 1981) or tetraethylammonium (TEA) (KOKETSU, 1958; MAENO *et al.*, 1982) were rarely recorded in PC-Ringer. Relatively slow development of facilitatory presynaptic effect found with PC (15–30 min to reach the quasi-equilibrium state) suggested that PC acts intracellularly as does 4AP. It might be concluded from these analogies that PC is a chemical potentiator or accelerator equivalent to 4AP.

Contrarily, considerable discrepancies in the presynaptic effects have been noted between NA and the release accelerators such as 4AP, PC, and TEA. While accelerators all prolonged the nerve terminal action potential and synaptic delay (MOLGÓ *et al.*, 1977; Maeno, unpublished), NA was devoid of such actions (KUBA, 1970). NA manifested its effect *via* α -adrenoreceptor (KUBA, 1970) whereas PC did not act at this receptor (BLABER and GALLAGHER, 1971). Finally, direct stimulation of the nerve terminal in the presence of tetrodotoxin clearly disclosed an important difference between the actions of accelerators and NA. The accelerators markedly increased the transmitter output by extending upward the stimulus intensity *versus* EPP amplitude relation (LUNDH, 1978). On the contrary, NA simply shifted the intensity-EPP curve to the left (KUBA and TOMITA, 1971). These suggest that NA does not act as an accelerator.

To explain the action of chemicals which accelerate remarkably the tetanic rundown of EPPs, MAENO *et al.* (1981) have postulated two types of evoked release of transmitter. Under ordinary conditions, the transmitter output is proportional to the amount of Ca-bound X (Ca_4X); *i. e.*

where K_{Ca} is the dissociation constant of Ca and P_{Ca} is the maximal P anticipated when all Xs are saturated with Ca. The analysis of NA actions by KUBA and TOMITA (1971) strongly suggests that NA modulates the regular evoked release; NA increases P_{Ca} without affecting K_{Ca} . This was confirmed by studying Ca

versus release relationship in the present paper. As shown in Fig. 1B, modified Lineweaver-Burk plot of the Ca data made it clear that low level potentiation of the EPP with 50 μM NA was attributed entirely to corresponding increase in P_{Ca} from 0.11 to 0.14; the value of K_{Ca} (0.57 mM) remained unaltered. On the other hand, both the apparent K_{Ca} and P_{Ca} were found to increase in the cases of 4AP and PC.

By combining with Ca_4X , accelerator (A) such as 4AP and PC enhances the efficacy of transmitter release resulting in profound augmentation of the parameter of evoked release from P_{Ca} to P_{A} ;

where K_{A} is the dissociation constant of A. Although Lineweaver-Burk plot of the PC data yields a linear relation, that of the NA data is independent of the NA concentration over a wide range ($[\text{NA}]^{-1} = 1 - 100 \text{ mM}^{-1}$). This suggests that the presynaptic action of NA cannot be explained from the above scheme. The accelerated evoked release by PC found in the present paper ($K_{\text{A}} = 35 \mu\text{M}$ and $P_{\text{A}} = 0.76$) was somewhat less than that of 4AP ($K_{\text{A}} = 8.5 \mu\text{M}$ and $P_{\text{A}} = 0.92$; MAENO *et al.*, 1981), but it was considerably more potent than TEA ($K_{\text{A}} = 0.5 \text{ mM}$ and $P_{\text{A}} = 0.90$; MAENO *et al.*, 1982). Thus, the present data reveal that measurement of the fractional release provides an important clue in determining the mechanisms of actions of various release modifying agents.

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