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Cocaine and desipramine antagonize the clonidine-induced inhibition of [^{3}H]-noradrenaline release from the rat cerebral cortex

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Imidazolines like clonidine are known to reduce the stimulation-evoked [^{3}H]-noradrenaline ([^{3}H]-NA) overflow from noradrenergic nerve endings through activation of presynaptic alpha-adrenoceptors, which mediate a negative feed-back mechanism in the peripheral as well as in the central nervous system (CNS) (Langer, 1977; Starke, 1977).

In the perfused cat spleen the inhibition of the stimulation-evoked release of [^{3}H]-NA by the imidazoline oxymetazoline is antagonized by two inhibitors of neuronal uptake: amphetamine and cocaine (Dubocovich, Langer & Moret, 1979). Consequently it was considered of interest to investigate a similar type of interaction in the CNS.

Male rats were killed by decapitation and slices prepared from the cerebral cortex were labelled *in vitro* by incubation with (±)-[^{3}H]-NA (0.5 μM). Slices were then superfused with Krebs solution and the release of [^{3}H]-NA was elicited by a 2 min period of electrical stimulation at a frequency of 3 Hz. In the controls the fraction of total tissue tritium released (FR) by the first period of stimulation (S_1) was: 3.41 ± 0.21 ($\times 10^2$) ($n = 23$). The ratio of FR between two consecutive stimulation periods S_2/S_1 was: 0.90 ± 0.05 ($n = 6$). Under these experimental conditions the release of [^{3}H]-NA was found to be entirely calcium-dependent. Clonidine reduced the stimulation-evoked [^{3}H]-NA release in a concentration-dependent manner. The inhibition of [^{3}H]-transmitter release was: 33.8 + 7.5% ($n = 7$); 57.1 ± 5.3% ($n = 5$) and 65.5 ± 4.4% ($n = 5$) for 0.03, 0.1 and 1 μM of clonidine respectively.

When cocaine (10 μM) was present in the superfusion medium throughout the experiment, the value of FR in S_1 was increased to: 7.44 ± 0.56 ($\times 10^2$) ($n = 12$, $P < 0.001$ when compared with the control values) and the ratio between two consecutive stimulation periods S_2/S_1 was: 1.06 ± 0.07 ($n = 3$). In the presence of cocaine (10 μM) the inhibition of [^{3}H]-NA release obtained with clonidine (0.1 and 1 μM) was only 24.2 + 7.9% ($n = 6$) and 14.8 ± 10.7% ($n = 4$) respectively. These effects were significantly smaller ($P < 0.01$) than those obtained in the absence of cocaine.

When desipramine (DMI, 0.1 μM) was present in the superfusion medium throughout the experiment the value of FR in S_1 was 5.18 ± 0.27 ($\times 10^2$) ($n = 24$, $P < 0.001$ when compared with the control values) and the ratio S_2/S_1 was: 1.21 ± 0.09 ($n = 5$). In the presence of DMI (0.1 μM) the inhibition of [^{3}H]-NA release obtained with clonidine (0.1 and 1 μM) was: 27.1 ± 5.8% ($n = 5$) and 38.2 ± 7.1% ($n = 4$) respectively. These reductions were significantly smaller ($P < 0.01$) than those observed in the absence of DMI. It is of interest to note that in the controls the alpha-adrenoceptor antagonist phentolamine (1 μM), significantly increased the stimulation-evoked [^{3}H]-NA release (S_2/S_1: 3.85 ± 0.77, $n = 6$, $P < 0.01$ when compared with controls). In the presence of DMI (0.1 μM) throughout the experiment phentolamine (1 μM), added before S_2 produced an increase of similar magnitude to that obtained in the absence of DMI (S_2/S_1: 3.61 ± 0.53, $n = 4$).

When neuronal uptake of (±)-[^{3}H]-NA (0.05 μM) was studied in rat cerebral cortex slices it was found to be inhibited by 80.5%, $n = 4$ and 81.0%, $n = 4$ in the presence of DMI (0.1 μM) and cocaine (10 μM) respectively. The increase in the stimulation-induced [^{3}H]-NA overflow obtained in the presence of cocaine and DMI is most probably due to the inhibition of NA uptake. Under our experimental conditions the antagonism by DMI of the clonidine induced inhibition of neurotransmission cannot be attributed to a blockade of presynaptic alpha-adrenoceptors by DMI because the facilitating effect on [^{3}H]-NA release by phentolamine was not modified in the presence of this drug.

It is concluded that an increase in the concentration of NA in the synaptic gap as obtained when neuronal uptake is inhibited may explain the decreased effectiveness of clonidine in reducing nor-

adrenergic neurotransmission in the CNS as already reported in the peripheral nervous system (Starke & Altmann, 1973; Dubocovich, *et al.*, 1979). Medgett, McCulloch & Rand (1978) demonstrated that an increase in the concentration of NA in the synaptic gap may shift the effects of clonidine from agonist to antagonist activity on presynaptic alpha-adrenoceptors. The fact that DMI antagonizes the inhibitory effect of clonidine on central noradrenergic neurotransmission could be relevant to the attenuated hypotensive effect observed for this imidazoline when administered in association with some antidepressants in spontaneously hypertensive rats (Dadkar, Dohadwalla & Bhattacharya, 1978) and in man (Briant, Reid & Dollery, 1973).

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Inhibitory presynaptic effects of hydralazine on noradrenergic nerve terminals of the rat tail artery

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It has been shown previously that the effects of hydralazine (HYD) on arterial smooth muscle are modulated by the release of ATP and/or adenosine from sympathetic nerve terminals (Worcel, 1978; Worcel & Saïag, unpublished). Nerve released purines have been implicated in an inhibitory feedback regulation in sympathetic terminals (Verhaeghe, Vanhoutte & Shepherd, 1976; Enero & Saïdman, 1977). In consequence it appeared interesting to explore the possibility of the existence of a presynaptic interaction between HYD and purines.

The preparation used was the rat tail artery already described. In a first series of experiments we have studied the vasoconstrictor responses of the proximal segments of the artery induced by field stimulation. Indeed, we have observed that the contractile response of the proximal segments of innervated arteries from normotensive Wistar rats, induced by exogenous agonists (phenylephrine, serotonin, lysine-vasopressin) was practically not affected by concentrations of HYD as high as 1 μM (Worcel, 1978). Conversely, the contractions induced by field stimulation were sizeably inhibited by HYD (0.3 μM and 3 μM). In order to confirm the existence of an HYD presynaptic effect, we studied the actions of the drug on tritium efflux-stimulation induced (S.I) from arteries loaded with [^{3}H]-noradrenaline. Indeed, HYD caused an inhibition of the S.I tritium efflux, which reached a plateau effect very rapidly, after 5 min of HYD superfusion. The reduction of fractional release induced by HYD is dose-dependent. The threshold of this presynaptic response is low, 30 nM produces a 30% reduction of S.I efflux. The dose-effect curve for the inhibitory presynaptic effect is extended, the maximal action of HYD not being obtained at 3 μM.

We have observed that theophylline (0.5 mM) potentiates the postsynaptic (smooth muscle) effects of HYD (Worcel & Saïag, unpublished). Conversely, theophylline appears to be ineffective presynaptically since a concentration of 0.5 mM did not alter significantly the inhibitory effect of HYD on the S.I tritium efflux from the rat tail artery.

In conclusion, the present results indicate that HYD has in addition to its action on vascular smooth muscle, a very marked effect on sympathetic nerve terminals. Nonetheless, the mechanism of this presynaptic inhibition appears to be different from the postsynaptic effect, given the much shorter delay, the