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[^{3}H]SPIROPERIDOL BINDING IN NORMAL AND DENERVATED CAROTID BODIES

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Specific dopamine receptors were studied in freshly dissected, unhomogenized rabbit carotid bodies incubated in [^{3}H]spiroperidol. Total binding and non-specific binding were determined in the absence and presence of 0.2 μM (+)-butaclamol, respectively. Specific binding in normal carotid bodies incubated at near saturating concentrations (0.38 nM) was 1.63 ± 0.58 pmol/g of tissue. Chronic section of the carotid sinus nerve (14 days) resulted in a 64% reduction ($P < 0.05$) in specific binding. We conclude that the majority of specific dopaminergic receptors are located on carotid sinus nerve afferent terminals.

The carotid body is a chemosensory organ which reflexly influences heart rate, vasoresistance and pulmonary ventilation. Changes in blood pO_2, pCO_2 and pH are translated by this organ into appropriate patterns of chemoreceptor discharge on the carotid sinus nerve (see ref. 3). However, the precise cellular and biochemical mechanisms involved in this chemosensory transduction process remain to be fully elucidated.

The parenchyma of the carotid body is composed of lobules, or glomeruli, of catecholamine-containing (type I) cells which receive synaptic terminations from fibers of the carotid sinus nerve [4, 5, 15, 17]. The type I cell/nerve terminal complex is enveloped by processes of sustentacular, or type II, cells. Blood flow through the carotid body utilizes an extensive network of fenestrated capillaries and sinusoids, which penetrate the connective tissue stroma surrounding the glomeruli [23].

The role of catecholamines in the carotid body has been investigated in numerous pharmacological, physiological and biochemical studies (see refs. 8 and 9). The actions of exogenously applied dopamine and dopaminergic agonists on chemosensory activity has received considerable attention in recent years. These studies have shown that dopamine initiates dose-dependent changes in receptor potential and frequency of chemoreceptor discharge [6, 16, 18–20]. It remains unclear, however, whether the pharmacological effects of dopamine are mediated via its direct action on the afferent terminals, or indirectly via the type I or other cells. In addition, because carotid chemoreceptor discharge is sensitive to blood

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flow [7, 14], interpretations from the in vivo effects of dopamine are complicated by possible accompanying and uncertain vascular changes [10]. Thus, while the existing evidence points to an important role for dopamine in chemosensory transduction, an understanding of the functional role of this substance would be considerably enhanced by a demonstration of the location and nature of dopaminergic receptors in this tissue. We report here the results of our study using the dopaminergic radioligand, [^{3}H]spiroperidol [24].

Tissues from two groups of rabbits were used in this study. In one group, the carotid sinus nerves were bilaterally resected under pentobarbital anesthesia 14 days prior to the binding assay; the other group included normal, unoperated animals. Carotid bodies were removed for assay from pentobarbital-anesthetized animals and cleaned of connective tissue in ice-cold modified Tyrode's solution [1]. Each carotid body was placed in a vial containing 1.5 ml of Tyrode's with 0.1–1.45 nM [^{3}H]spiroperidol (specific activity = 25.1 Ci/mmol, New England Nuclear) and incubated for 20 min in a waterbath-shaker at 37°C. The tissues were then washed for 6 min in 10 ml of ice-cold Tyrode's solution. Each carotid body was weighed on a Cahn electrobalance fitted with a humidified chamber to prevent drying of the tissues. Carotid bodies were combusted in a sample oxidizer (Packard Model 306, tritium recovery 99+%) before counting in a liquid scintillation spectrometer (Packard Model 3385, E^2/B approx. 140 with Oxifluor–H_2O, New England Nuclear). [^{3}H]Hexadecane standards were used to construct quench correction curves through sample combustion and counting. Total binding and non-specific binding were determined in the absence and presence, respectively, of 0.2 μM (+)-butaclamol (Ayerst Research Laboratories) [22], the difference between total and non-specific binding being defined as the specific (displaceable) binding. The data are expressed as pmol [^{3}H]spiroperidol bound/g of tissue ($\pm$ S.E.) and the data were evaluated using Student's t-test.

Our results indicate the presence of high affinity dopaminergic receptors in the rabbit carotid body. Time course studies showed that specific binding of [^{3}H]spiroperidol reached a plateau after 20 min of incubation and Scatchard analysis of specific binding yielded a K_D = 0.16 nM and a B_{max} = 2.15 pmol/g of tissue.

Fig. 1 shows the total, non-specific and specific [^{3}H]spiroperidol binding for normal and denervated rabbit carotid bodies. Specific binding in normal carotid bodies was 1.63 $\pm$ 0.58 pmol/g of tissue, and in denervated tissue was 0.58 $\pm$ 0.28 pmol/g (statistically different at $P < 0.05$). Thus, chronic transection of the carotid sinus nerve reduced specific binding by 64%. These data suggest that the majority of specific dopaminergic receptors in rabbit carotid body are associated with the terminals and/or fibers of the carotid sinus nerve. When these findings are considered in light of the reported pharmacological effects of dopamine on the receptor potential and chemoreceptor discharge from rabbit carotid body [6, 18, 20], the results strongly suggest the presence of high affinity dopaminergic receptors on the afferent terminations of this nerve.

Fig. 1 also shows that the non-specific binding of [^{3}H]spiroperidol in both normal and denervated carotid bodies is large in comparison to specific binding. This result

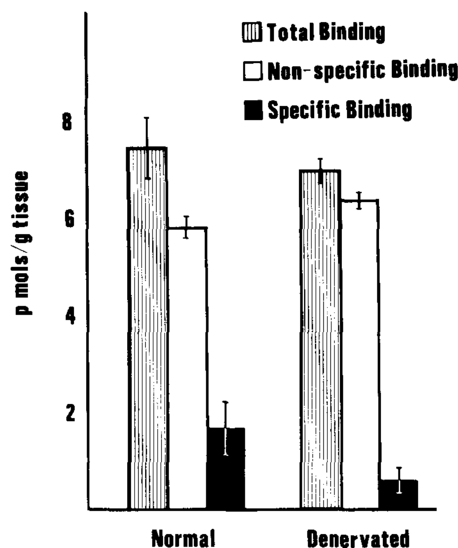

Fig. 1. Total, non-specific and specific [^{3}H]spiroperidol binding in normal and chronically denervated (carotid sinus nerve transection) rabbit carotid bodies. [^{3}H]Spiroperidol concentration was 0.38 nM (near saturating). Total and non-specific binding were determined in the absence and presence, respectively, of 0.2 μM (+)-butaclamol, the difference between the two being defined as specific binding. Values are expressed in pmol [^{3}H]spiroperidol bound/g tissue ± S.E. Total binding exceeded non-specific binding in both normal (n = 14, $P < 0.025$) and denervated (n = 40, $P < 0.05$) carotid bodies, and specific binding in denervated organs was reduced by 64% ($P < 0.05$).

can be attributed in part to the high degree of lipid solubility of spiroperidol [22], combined with the unavoidable presence of adipocytes in intact carotid body preparations. With respect to *specific* binding in intact tissue, it is conceivable that intracellular accumulation via catecholamine uptake mechanisms might have influenced our kinetic data. However, several findings would tend to argue against this possibility: (1) neuroleptic drugs similar to spiroperidol (e.g. haloperidol) do not influence dopamine uptake at the concentrations used in our study [22]; (2) the localization of specific spiroperidol binding in intact rat striatal tissue is comparable to that found for homogenates [12, 13]; and (3) inhibitors of dopamine uptake display a low affinity for [^{3}H]dopamine binding sites in striatal membranes [2].

The precise location of the dopaminergic receptors remaining in denervated carotid bodies cannot be determined from our present data. These receptors could possibly be associated with vascular elements, sympathetic nerve endings or other non-glomerular structures. However, one interesting possibility is that dopaminergic receptors might be located on the type I cells themselves. Such receptors would then be analogous to the autoreceptors which influence dopamine metabolism in striatal neurons [11, 21, 25]. We are currently investigating this possibility by examining the effects of apomorphine and other dopaminergic agonists on the synthesis and release of catecholamines from normal and denervated rabbit carotid bodies.

In conclusion, numerous studies in recent years have suggested that dopamine is a

likely candidate for neurotransmitter or neuromodulator between type I cells and their afferent nerve terminals in the carotid body. The results of the present study are consonant with this hypothesis and, in addition, suggest the possible involvement of dopaminergic autoreceptors in local feedback and regulation of chemoreceptor mechanisms.

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