PHARMACOLOGICAL MODULATION OF BRAIN NICOTINIC BINDING SITES

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It is well documented that receptors for neurotransmitters, hormones and drugs are regulated by homeostatic mechanisms which serve to compensate for changes in the amount of agonist or antagonist to which they are exposed. Such changes in receptor sensitivity can be measured by radioreceptor ligand-binding assays, by measurements of biochemical events associated with receptor activation (e.g. metabolism of phosphoinositides, activity of adenylate cyclase, ion fluxes) or by recording distal events both in vitro and in vivo (e.g. the firing of specific neurons, certain behavioral effects). In general, a decreased neurotransmitter input, as obtained with presynaptic chemical or mechanical denervation or by chronic receptor blockade with an antagonist, leads to hypersensitivity. Conversely, an increased neurotransmitter input, via a direct or indirect interaction by agonists with the receptors, triggers the development of hyposensitivity. This pattern of receptor modification has been observed, for example, with beta-adrenoceptors, dopamine receptors and cholinergic muscarinic receptors (Daly et al. 1980; Creese and Sibley, 1981; Schwartz et al. 1983). The regulation of brain muscarinic cholinergic receptors, for example, has been studied extensively. Chronic treatments with direct acting agonists, such as oxotremorine or carbachol, or with indirect agonists, such as acetylcholinesterase inhibitors, cause a hyposensitivity of muscarinic receptors. This alteration of muscarinic receptor sensitivity is manifested in a decrease in receptor density measured by radioligand binding (Costa et al. 1982; Ho and Hoskins, 1987; Russell and Overstreet 1987), a decrease in muscarinic receptor-mediated cellular responses (Olianas et al. 1984; Costa et al. 1986a) and a decrease of various behavioral effects elicited by cholinergic agonists (Costa et al. 1982;

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Russell and Overstreet, 1987). Conversely, repeated treatments with muscarinic antagonists cause an increase in the density of muscarinic receptors and a subsensitivity and supersensitivity, respectively, to the behavioral effects of muscarinic antagonists and agonists (Majocha and Baldessarini, 1984).

The identification in brain tissue of binding sites for various nicotinic ligands (Clarke, 1986; Wonnacott, 1987; Table 1)

Table 1. Ligands used to label nicotinic receptors in brain tissue

^{3}H-Nicotine
^{3}H-Acetylcholine
^{3}H-Methylcarbamylcholine
^{3}H-Dihydro-beta-erythroidine
^{3}H-Tubocurarine
^{125}I-alpha-Bungarotoxin

presumably related to nicotinic acetylcholine receptors, has led to the study of their pharmacological modulation following repeated exposure to cholinergic drugs or other compounds. In this chapter, I have summarized our current information on such modulation particularly following *in vivo* administration of anticholinesterase compounds and nicotine.

IN VIVO MODULATION OF BRAIN NICOTINIC BINDING SITES BY ANTICHOLINESTERASE AGENTS

Inhibitors of acetylcholinesterase (AChE), such as organophosphates and carbamates, are widely used as pesticides and have limited application in pharmacotherapy. Toxic effects resulting from acute exposure are due to accumulation of acetylcholine in the synaptic cleft with consequent hyperstimulation of cholinergic receptors. Symptoms of intoxication are usually divided into nicotinic (e.g. muscle fasciculation), muscarinic (e.g. increased salivation, diarrhea)

and central nervous sytem effects (Costa, 1987). Although these central effects are possibly due to activation of both muscarinic and nicotinic cholinergic receptors, little attention has been devoted, so far, to the role of nicotinic receptors in acute anticholinesterase poisoning. The muscarinic receptor antagonist atropine represents the treatment of choice for anticholinesterase intoxication; recent studies, however, have shown that the addition of a nicotinic cholinergic antagonist such as mecamylamine to the antidotal "cocktail" (consisting of the oxime 2-PAM and atropine) can in some cases increase the protection and/or recovery from acute organophosphate poisoning (Harris et al, 1980; Chiou et al. 1986). Although some of this protective effect might be due to an action of nicotinic antagonists on peripheral ganglia, a central action is also possible. For example, it has been shown that the antinociceptive effect of diisopropylfluorophosphate (DFP) and physostigmine is antagonized by scopolamine, a muscarinic antagonist, as well as by the nicotinic antagonist mecamylamine (Zorn,1983).

Recently, there has been a revival of interest in the effects of chronic exposure to anticholinesterase compound. Already in the early 1950s, it had been observed that animals fed the organophosphorus insecticide parathion had clearly diminished signs of toxicity after two months on the diet (Barnes and Denz 1951). This "tolerance" to the toxic effects of organophosphates following repeated exposures has been observed by several investigators and has been recently reviewed (Costa et al. 1982; Ho and Hoskins, 1987; Russell and Overstreet, 1987). Brodeur and DuBois (1964) first observed that animals, made tolerant to the toxicity of the anticholinesterase insecticide disulfoton, were also resistant to the lethal effect of the cholinergic agonist carbachol. This and other studies showing subsensitivity to various effects of cholinergic muscarinic agonists (reviewed by Costa et al. 1982), led to the formulation of the hypothesis that tolerance to organophosphate toxicity is due, at least in part, to an alteration of cholinergic receptors (Russell et al. 1975). In the last ten years several laboratories have indeed shown that repeated exposure to a variety of organophosphates cause a down regulation of muscarinic cholinergic receptors in brain as well as

in peripheral tissues (reviewed by Costa et al. 1982; Ho and Hoskins, 1987; Russell and Overstreet, 1987).

Although the literature on the role of nicotinic cholinergic receptors in organophosphate tolerance is more limited, few recent studies have investigated possible alterations of brain nicotinic binding sites following repeated exposure to anticholinesterases. Overstreet et al.(1974) first reported that subchronic treatments with DFP rendered the animals subsensitive to the effects of nicotine on free operant responding, and suggested that brain nicotinic receptors might be altered.

Early studies had also shown that in rats repeatedly exposed to a cholinesterase inhibitor, the toxicity of the nicotinic antagonists decamethonium and d-tubocurarine was increased (McPhillips, 1969) which would be consistent with a decrease of nicotinic receptor density in the neuromuscular junction. A decrease of nicotinic receptors in the diaphragm of neostigmine-tolerant animals has been reported (Chang et al. 1973). Similarly, Gupta et al. (1986) have found a decrease of ^{3}H-acetylcholine binding in the diaphragm of rats made tolerant to DFP. Thus, a down-regulation of nicotinic receptors at the neuromuscular junction might be involved in the observed attenuation of the peripheral nicotinic signs of organophosphate poisoning.

Only recently some studies have investigated the effects of repeated administration of cholinesterase inhibitors on brain nicotinic binding sites (Table 2). Schwartz and Kellar (1983, 1985) found a decreased binding of ^{3}H-acetylcholine in brain of rats repeatedly treated with DFP. The decrease ranged between 19 and 37%, depending on the brain area, and was due to a decrease in receptor density with no change in affinity.

Using ^{3}H-nicotine as a ligand, Costa and Murphy (1983) showed that rats made tolerant to the organophosphorus insecticide disulfoton presented a decrease of binding in forebrain. This alteration was due to a 40% decrease in the density of the high affinity sites, with no changes in affinity and in any parameter of the low affinity site. This biochemical alteration was paralleled by a reduction in the antinociceptive effect of nicotine (Tripathi et al. 1982) in animals tolerant to disulfoton.

Costa and Murphy (1983) concluded, therefore, that the high affinity ^{3}H-nicotine site, whose binding parameters are similar to those of ^{3}H-acetylcholine, probably represent a true cholinergic nicotinic receptor. The nature and role of the low affinity site, however, remains to be elucidated. In a subsequent study, Costa and Murphy (1985) confirmed a 30% decrease of ^{3}H-nicotine high affinity binding in forebrain from DFP-tolerant mice. Decreases in the high affinity component of ^{3}H-nicotine binding, ranging from 40 to 56% have also been reported by Lim et al. (1987a) in DFP-tolerant rats. Two studies have measured binding of ^{125}I-alpha-BTX in brain of DFP-treated rats. Larsson et al. (1987) observed a decrease of ^{125}I-alpha-BTX binding in cerebral cortex but not in hypothalamus or hippocampus, while Overstreet (unpublished observation cited in Russell and Overstreet, 1987) did not find any alteration in alpha-BTX binding.

Table 2. In vivo regulation of brain nicotinic binding sites by anticholinesterase agents.

Anticholinesterase	Ligand	Effect On Binding	Ref.
Disulfoton	^{3}H-nicotine	Decrease	Costa and Murphy, 1983
DFP	^{3}H-acetylcholine	Decrease	Schwartz and Kellar, 1983; 1985
	^{3}H-nicotine	Decrease	Costa and Murphy, 1985;
	^{3}H-nicotine	Decrease	Lim et al. 1987a
	^{125}I-alpha-BTX	Decrease	Larsson et al. 1987
	^{125}I-alpha-BTX	No change	Russell and Overstreet, 1987

The observed changes in ^{3}H-nicotine and ^{3}H-acetylcholine binding following repeated anticholinesterase exposure could be due to a direct interaction of the cholinesterase inhibitors with nicotic cholinergic receptors. Indeed, several organophosphates, as well as carbamates have been shown to interact directly with the nicotinic receptor in Torpedo (Eldefrawi et al. 1982; Sherby et al. 1985). Several lines of evidence, however, argue against such a possibility in case of brain nicotinic binding sites. First, a series of eight organophosphates, some of which inhibited ^{3}H-acetylcholine binding in Torpedo (Eldefrawi et al. 1982), were unable to inhibit ^{3}H-nicotine binding in rat forebrain in vitro, when present at concentrations as high as 10^{-4}M (Costa, unpublished observation). Second, administration to rats of a near lethal dose of disulfoton, which inhibited brain AChE by 99%, did not cause any alteration of ^{3}H-nicotine binding (Costa and Murphy, 1983). Similarly, a single dose of DFP did not alter ^{3}H-nicotine binding in rat brain areas (Lim et al. 1987a).Thus, the alterations of ^{3}H-nicotine and ^{3}H-acetylcholine binding observed following repeated exposure to AChE inhibitors appear to be the consequence of prolonged receptor stimulation by endogenous acetylcholine, which accumulates in the synaptic cleft (Lim et al. 1987b). The decreased sensitivity to the antinociceptive effect of nicotine (Costa and Murphy, 1983) appears to correlate with the observed decrease in receptor density. However, this would be in contrast to the subsensitivity to other effects of nicotine observed in nicotine-tolerant animals, where an increase in receptor density is present (Marks et al. 1983; 1985; 1986). The concurrent decrease of muscarinic receptors could explain the observed subsensitivity to nicotine-induced antinociception if this effect were due to an interaction of nicotine with muscarinic sites. However, nicotine antinociception is not antagonized by atropine, while it is readily blocked by mecamylamine, pempidine and neosurugatoxin (Tripathi et al. 1982; Yamada et al. 1986). Thus, functional and behavioral correlates of decreased nicotinic binding sites following repeated anticholinesterase exposure warrant further investigations, particularly in the light of the contrasting results obtained with chronic nicotine treatments.

IN VIVO MODULATION OF BRAIN NICOTINIC BINDING SITES BY NICOTINE.

Several studies have examined the effects of repeated administration of nicotine on brain nicotinic receptors, particularly in an attempt to determine whether alteration of these sites would play a role in the development of tolerance to nicotine. As shown in Table 3, contrary to what was initially expected, following chronic nicotine exposure, most investigator found an increase in brain nicotine receptors, measured by both ^{3}H-nicotine and ^{3}H-acetylcholine binding, (Marks et al. 1983; 1985; Schwartz, Kellar 1983; 1985; Morrow et al. 1985; Ksir et al. 1985). Repeated treatments with the nicotinic agonist cytisin also increased ^{3}H-acetylcholine binding in rat cerebral cortex (Schwartz and Kellar, 1985). These changes were always due to an increase in the density of nicotinic binding sites with no changes in affinity. A few studies, however, did not confirm these findings. Sershen et al. (1982), Benwell and Balfour (1985) and Abood et al. (1987) did not find any alteration in ^{3}H-nicotine binding, while Falkeborn et al. (1981) and Nordberg et al. (1985a) reported decreases of ^{3}H-tubocurarine or ^{3}H-nicotine binding following prolonged administration of nicotine. The reasons for these discrepancies are not apparent at the moment. A series of studies also examined the effect of repeated administration of nicotine to pregnant rats on brain nicotinic receptors in the developing pups. Hagino and Lee (1985) administered nicotine to rats from day 15 of gestation. ^{3}H-nicotine binding was increased in the brain of fetuses on day 18 and 20 of gestation, as well as in the brain of the dams. Similar results were obtained in newborn rats from nicotine-treated dams (Sershen et al. 1982). A more detailed study was recently conducted by Slotkin et al. (1987). Pregnant rats were exposed to nicotine either by daily injections or by infusion from day 4 to day 20 of pregnancy. ^{3}H-nicotine binding was increased in fetal whole brain on gestational day 18. The effect of prenatal nicotine exposure became most prominent during the second postnatal week and was most persistent in the cerebellum with only minor effects in the cerebral cortex. Since the cerebellum is the primary target for disruption of cellular development by prenatal nicotine exposure, these

investigators suggested that the observed effect are consistent with a primary teratologic action of the drug rather than direct

Table 3. Effect of chronic nicotine exposure on brain nicotinic binding sites

Ligand	Effect on Binding	Reference
^{3}H-tubocurarine	Decrease in midbrain No change in cortex and hippocampus	Falkeborn et al. 1981
^{3}H-nicotine	Increase in hippocampus Decrease in cortex	Nordberg et al. 1985
^{3}H-nicotine	No change	Benwell and Balfour, 1985
^{3}H-nicotine	No change	Abood et al. 1987
^{3}H-nicotine	No change	Sershen et al. 1982
^{3}H-nicotine	Increase in all brain areas	Marks et al. 1983; 1985; 1986
^{3}H-nicotine	Increase in striatum	Fung and Lau, 1987
^{3}H-acetylcholine	Increase in all brain areas	Schwartz and Kellar 1983; 1985
^{3}H-acetylcholine	Increase in hippocampus	Morrow et al. 1985
^{3}H-acetylcholine	Increase in cortex	Ksir et al. 1985
^{125}I-alpha-BTX	Increase in midbrain and hippocampus	Marks et al. 1983
^{3}H-nicotine[a]	Increase	Sershen et al. 1982
^{3}H-nicotine[a]	Increase	Hagino and Lee, 1985
^{3}H-nicotine[a]	Increase in cortex and cerebellum	Slotkin et al. 1987

[a]Nicotine was given to pregnant rats and ^{3}H-nicotine binding was measured in the developing pups.

effects on development of ^{3}H-nicotine receptors (Slotkin et al. 1987).

The increased binding observed following repeated exposure to a cholinergic nicotinic agonist, such as nicotine, is unusual. As mentioned previously, chronic treatment with agonists normally decreases, while antagonists increase, the density of neurotransmitter receptors. Furthermore, it has been shown that stimulation of brain nicotinic receptors by endogenous acetylcholine (through inhibition of acetylcholinesterase) decreases the density of nicotinic binding sites. The current view is that chronic treatment with nicotine results in a shift of distribution of receptor conformations to the desensitized, and therefore, inactive state (Marks et al. 1986). This could lead to the impossibility of receptor activation by the endogenous agonist and, therefore, is equivalent to the treatment with an antagonist. However, Schwartz and Kellar (1985) reported that repeated treatments of rats with the centrally active nicotinic antagonists mecamylamine and dihydro-beta-erythroidine did not alter ^{3}H-acetylcholine binding in cerebral cortex. Moreover, neither drug was capable of preventing the increase in ^{3}H-acetylcholine binding induced by nicotine. An explanation for these findings is that these antagonists act on a site distinct from the agonist recognition site (Schwartz and Kellar (1985). Further studies are needed to substantiate this hypothesis.

A very important aspect of receptor modulation is the correlation of the observed biochemical alterations with changes in the behavioral effects of nicotine. Ksir et al. (1985) found that repeated administration of nicotine caused sensitization to behavioral stimulation by nicotine, i.e. the effect of nicotine was higher in nicotine-pretreated rats than in controls. There was some correspondence between the enhanced ^{3}H-acetylcholine binding in the cortex and the behavioral effects of nicotine, although a clear dose-response was not observed in the biochemical studies (Ksir et al. 1985). These authors concluded that the enhanced behavioral effect of nicotine after repeated exposure is likely linked to the observed increase in brain nicotinic cholinergic receptors. This would be in agreement with what is normally observed with other receptor systems, and with the

results obtained following chronic anticholinesterase exposure, where a decrease in brain ^{3}H-nicotine binding was paralleled by a decrease in the effect of nicotine on nociception (Costa and Murphy, 1983). On the other hand, a series of studies by Marks and Collins (Marks et al. 1983; 1985; 1986) have investigated the relationship between the development of tolerance to various behavioral effects of nicotine and changes in ^{3}H-nicotine binding. Tolerance did develop to the effects of nicotine on heart rate, body temperature, Y-maze crosses and Y-maze rears, but not to its effects on respiratory rate or startle response. Tolerance to nicotine was maximal after four days of drug treatment and paralleled the changes in ^{3}H-nicotine binding. Furthermore, the time-course for the loss of tolerance closely resembled the rate of decrease of cortical ^{3}H-nicotine binding. Thus, for certain effects of nicotine there appears to be an association between the diminished effects of nicotine during tolerance and an increase in ^{3}H-nicotine binding sites.

IN VIVO MODULATION OF BRAIN NICOTINIC BINDING SITES BY CHOLINE AND OTHER COMPOUNDS

Choline, the precursor of acetylcholine, is known to be a weak muscarinic agonist. In <u>in vitro</u> binding studies choline has been found to inhibit the binding of ^{3}H-quinuclidinyl benzilate with IC_{50}s ranging from 1.3 to 2.5 mM (Palacios and Kuhar, 1979; Costa and Murphy, 1984). It has also been shown that choline can inhibit acetylcholine release, possibly activating M_2 muscarinic receptors (Kilbinger and Kruel, 1981), whereas it acts as a partial agonist at M_1 receptors regulating phosphoinositide metabolism (Costa et al. 1986b). <u>In vitro</u> binding studies have shown that choline can inhibit the binding of ^{3}H-nicotine to rat brain membranes with an IC_{50} of 240 μM (Costa and Murphy, 1984). In addition, choline has been shown to act as a partial agonist in stimulating the release of catecholamines from primary dissociated cultures of bovine adrenal medullary chromaffin cells by interacting with nicotinic receptors (Holz and Senter, 1981). A series of studies by Morley and collaborators (Morley et al. 1977;

Morley and Gabner, 1986; Morley and Fleck, 1987) have shown that dietary choline increases the density of ^{125}I-alpha-BTX binding sites in various brain areas. The effect was more pronounced in young rats than in aging animals. This increase in alpha-BTX binding occurred rapidly (within 24 h) whereas the effects of repeated nicotine administration were evident only after several days (Marks et al. 1985), and were reversible and dose-dependent. It is unlikely that this upregulation of alpha-BTX binding was due to an increased level of brain acetylcholine (Cohen and Wurtman, 1975), since as discussed above, this has been shown to cause a decrease or no change of ^{125}I-alpha-BTX binding (Larsson et al. 1987; Russell and Overstreet, 1987). It might be, therefore, due to a direct effect on nicotinic receptors, similar to that of nicotine, although the exact mechanism by which dietary choline produces an increase in the concentration of brain nicotinic receptors has yet to be determined. Interestingly, choline has been suggested as a possible endogenous ligand for brain nicotinic receptors (Polz-Tejera and Schmidt, 1983), although this possibility has been discounted by others (Perry et al. 1986).

Studies investigating the effects of other drugs or chemicals on brain nicotinic binding sites are scanty. Two studies have investigated the effects of chronic ethanol treatment on ^{3}H-nicotine binding. No changes were found in cerebral cortex of rats seven days after withdrawal of an 83 weeks treatment (Nordberg et al. 1985b). In vitro addition of ethanol did not alter ^{3}H-nicotine binding, but during a five month ethanol exposure, increased ^{3}H-nicotine binding was observed in rat hypothalamus and thalamus, while a decrease was present in the hippocampus (Yoshida et al. 1982). The mechanism responsible for such alterations and their relevance in the central effects of ethanol and in tolerance and dependence remain obscure.

Neonatal administration of estradiol to female rats has also been shown to increase ^{125}I-alpha BTX binding in the hypothalamus (Block and Billiar, 1979; Rodriguez-Sierra et al. 1982). Again, the significance of this finding is elusive.

IN VITRO MODULATION OF BRAIN NICOTINIC BINDING SITES

A few studies have examined the regulation of neural nicotinic receptors by cholinergic nicotinic agonists *in vitro*. Muscarinic receptors have been shown to be similarly regulated by agonists in *in vivo* and *in vitro* systems (Nathanson, 1987). On the other hand, studies on neuronal nicotinic receptors have yielded contrasting results. A decrease of ^{125}I-alpha-BTX binding has been reported in embryonic chick ciliary ganglion neurons following prolonged incubation with carbachol or nicotine (Messing, 1982). Similarly, chronic carbachol treatment was found to cause a functional down-regulation of nicotinic receptors (measured by $^{86}Rb^{+}$ fluxes) in PC12 cells (Robinson and McGee, 1983). On the contrary, no changes in the binding of ^{125}I-alpha-BTX or ^{3}H-bromoacetylcholine were found in chick retinal cells following prolonged incubation with carbachol or nicotine (Betz, 1982; Siman and Klein, 1983).

The limited number of *in vitro* studies available does not allow any conclusion on possible correlation between *in vivo* and *in vitro* systems. It is noteworthy, however, that no up-regulation of nicotinic binding sites has been observed *in vitro*.

CONCLUSION

It is apparent from this brief review that our knowledge on the *in vivo* pharmacological regulation of brain nicotinic binding site is still limited. The possible relevance of such binding sites in the mechanisms underlying tolerance and dependence to nicotine warrants further studies in this area. Further studies should investigate the dose and time relationship of the effects of repeated exposure of cholinesterase inhibitors and other nicotinic agonists on brain nicotinic receptors, as well as the effects of nicotinic antagonists. Other aspects that need particular attention include, for example, the role and pharmacological regulation of the low affinity binding sites for ^{3}H-nicotine, the regulation of the alpha-BTX site and the correlation between biochemical alterations and functional aspects of brain nicotinic

receptors. The latter includes not only studies aimed at determining in vitro/in vivo relationships of the effects of nicotine, but in particular, studies aimed at investigating the intracellular events which accompany the activation of nicotinic receptors in brain tissue. For example, some recent results suggest that activation of nicotinic receptors in bovine adrenal chromaffin cells stimulate the metabolism of phosphoinositides, increase intracellular calcium concentration and promote the translocation of protein kinase C (Eberhard and Holz, 1987). Translocation of protein kinase C induced by cholinergic agonists in PC12 cells has also been shown to be due to activation of nicotinic receptor which are linked to dihydropyrine-sensitive Ca^{2+} channels (Messing and Stevens, 1987). Preliminary studies in our laboratory, however, failed to evidentiate any stimulation of phosphoinositide metabolism in cerebral cortex slices by the nicotinic agonist 1, 1-dimethyl-4-phenyl-piperazinium (Balduini and Costa, unpublished observations). Studies aimed at establishing a relationship between binding and functional characteristics of the receptor will certainly improve our understanding of the physiological and pathological significance of the adaptive modifications of nicotinic receptors in nervous tissue.

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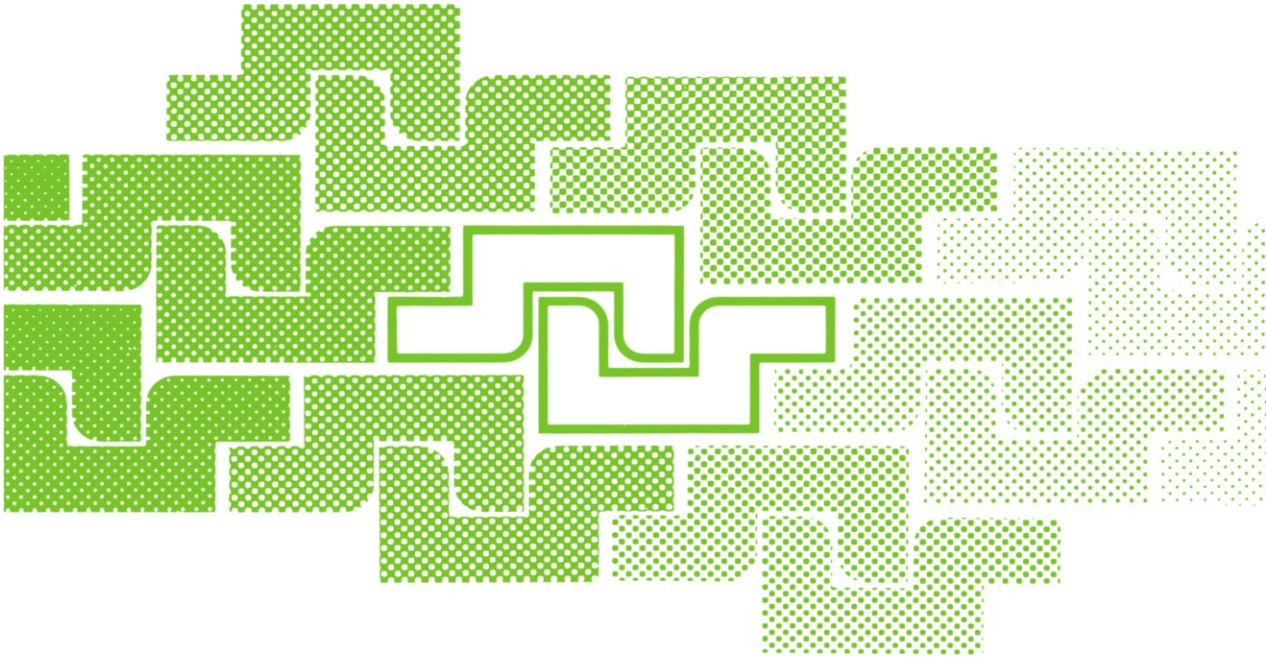

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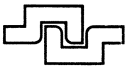

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