IV. DISCUSSION

Discussion

An analogy has been suggested between the synapses of peripheral antonomic ganglia and the synapse presents in SON and PVN. This analogy was first proposed by Walker (1957) on the basis of studies examining the effects of pharmacological doses of nicotine on ADH release. However, subsequent studies have failed to conclusively substantiate this contention. Bisset and Walker (1957) and Supek and Eisen (1956) from studies on the effects of nicotine and various ganglionic blocking drugs on ADH release, claimed that if a synapse existed at the SON, it was dissimilar in its pharmacological properties to synapses at autonomic ganglia. The major reason for their claim was the finding that the ganglionic blocking drug, hexamethonium, was ineffective in preventing the rise in ADH induced by nicotine. Schrier (1974) on the other hand found that nicotine administered into the carotid artery of hydrated anesthetized dogs did not increase ADH release, while an intravenous administration of the drug did result in release of ADH. He concluded that intravenous nicotine stimulated ADH release indirectly through a fall in blood pressure which it induced due to ganglionic blockade.

In the present study evidence is presented to show that nicotine induced the release of ADH and that this was due to a direct effect on the supraoptic neurohypophyseal system instead of an indirect

effect via change in systemic hemodynamics. On the basis of both this action of nicotine as well as the effects of ganglionic stimulants and blockers, it is suggested that the synapse at the SON is a cholinergic one with a nicotinic receptor, similar to that existing in autonomic ganglia. In addition, similarity between the SON and peripheral ganglia is suggested by the fact that both these structures are sensitive to A-II. Evidence is also presented with these studies to show that a sympathetic synapse exists at the SON where α-adrenergic agents stimulate the release of hormone while β-adrenergic agents have no effect on ADH release.

In the present study ADH release was measured by a very sensitive and reproducible radioimmunoassay for arginine vasopressin which I developed in our laboratory. This technique is more sensitive and reproducible than the bioassay used in the majority of the previous studies on the control of ADH release. In addition, direct measurement of hormone concentration is more specific for changes in ADH release than measurement of such parameters as urine flow, urine osmolality or free water clearance. The latter parameters, although they can be used to assess changes in ADH effects, are also affected by many other factors such as blood pressure, renal blood flow, and other hormones (prostaglandins).

All the in vivo experiments were conducted in conscious animals so that the nonspecific and confounding effects of the anesthetic

agents would not interfere with the drug's action. This is especially significant in the light of the recent demonstration that general anesthetics like ether and pentobarbitone alter the functional properties of the osmoregulatory system (Valtin and Braunwald, 1975).

In order to provide additional independent measurements on the control of the ADH release, experiments were done using a totally in vitro system. It was felt that exposing isolated SONH to various proposed stimulators and inhibitors of ADH release in an in vitro system would avoid the confusing effects of potential indirect mechanism for changing ADH release.

In the present study, acetylcholine (Ach) in both the in vitro and in vivo experiments increased ADH release. This is in agreement with the early studies of Pickford and her colleagues (1939, 1947) who found that Ach and acetylcholinesterase inhibitors produce an antidiuresis in experimental animals, and that Ach injected directly into the SON causes antidiuresis. In addition, the present studies are also in agreement with those of Bhargava et al. (1972) who have shown that Ach administered into the cerebral ventricles of dogs produces an increase in ADH level. This mutual agreement strongly suggests that Ach acts as a transmitter agent in a synapse in the SON.

Nicotine elicited a dose-dependent release of ADH in the presence of an elevated MABP. This finding is in contrast with Schrier's

studies (1974) in which he injected nicotine into the carotid artery of anesthetized dogs and failed to find an antidiuretic effect. However, with intravenous injection of nicotine an antidiuretic effect was found. Schrier (1974) attributed the antidiuretic response to intravenous injection to a fall in BP secondary to peripheral sympathetic blockade. The present in vivo studies suggest a direct effect of nicotine because ADH was released in spite of an increase in BP which would have been expected to inhibit ADH release. In support of the interpretation that nicotine increases ADH release independent of hemodynamic changes, is the finding, in the present studies, of in vitro release of ADH in response to nicotine.

In contrast to nicotine, McN-A-343-11, a murcarinic ganglionic stimulant, did not have an effect on ADH release either in in vivo or in vitro studies. These findings strongly suggest that the nature of the synapse at the SON cannot be murcarinic. This interpretation might appear to be in contrast with the findings of Bhargava et al. (1972) who found that higher doses of atropine administered into the cerebral ventricles of the dog significantly reduced the effect of Ach-induced release of ADH. Bhargava et al. (1972) concluded from these findings that the action of Ach is murcarinic in nature. However, it was entirely possible that the inhibitory action of atropine they discovered is similar to the known action of atropine

on the autonomic ganglia described by Marrazzi (1939). As ganglionic synapses in the peripheral autonomic nervous system are nicotinic and cerebral synapses are probably of a similar nature (Marazzi et al., 1951) it would seem unlikely that such murcarinic site would be present at the SON. Assuming then that the studies with Ach and nicotine (especially since the in vitro and in vivo results are in agreement) establish that a nicotinic cholinergic receptor is involved in the release of ADH, one can reasonably classify the SON as ganglionic synapse. The remainder of the studies presented here were then aimed at further characterization of the synapses that are present.

When A-II was infused into the carotid artery of the dog, it produced a slight but not significant release of ADH. In addition, it did not elicit a dose dependent release of ADH as opposed to the study of Malvin (1971). In the latter study a clear cut dose-dependent release of ADH could not be consistently reproduced. A possible explanation for the present findings could be that the increase in blood pressure caused by A-II inhibited ADH secretion significantly and caused an attenuation of a direct effect on ADH release by A-II. This presumption is supported by the in vitro experiments using SONH. Here A-II did increase the release of ADH significantly. Of interest is the fact that A-II has been shown to stimulate peripheral autonomic ganglia (Lewis and Reit, 1965). This finding along with the present studies lends further support to the concept of viewing the SON as synapse.

This was further clarified by studying the modification by ganglion blocking drugs of the effect of hypertonic NaCl to stimulate the release of ADH. Ganglionic blocking drugs, mecamylamine and pentolinium completely blocked the release of vasopressin caused by hyperosmotic stimulation with NaCl both in dogs and the SONH preparation. These results are at variance with those of de Vied and Laszlo (1967) who obtained no inhibition of osmotic stimulation with mecamylamine and those of Harris et al. (1969) who stated that 5 mg of pempidine injected intravenously into cats was ineffective in blocking ADH release secondary to hypertonic NaCl. In the former case, the dose of mecamylamine used was not given and hence it is difficult to interpret the findings. In the latter case it is possible that the intravenous dose was too low and an intracarotid injection might have been effective.

It has been shown that Ach releases catecholamine in the periphary (Douglas and Rukin, 1961) and centrally (Phillips et al., 1970). Vogt (1954) and Carlsson (1959) have reported that the hypothalamus is very rich in catecholamines. Bertler (1960) has mapped synthesizing and inactivating enzymes for mono amines in the central nervous system. Histochemical localization of the catecholamines has been accomplished by Furl et al. (1969). Carlsson et al. (1962) and Fuye (1965) reported that the SON and PVN are richly innervated with

adrenergic nerve terminals. Adrenoceptive drugs after intravenous administration have been shown to facilitate (Dearborn and Lasagna, 1952; Houch, 1961; Eranko and Karnonin, 1952) as well as inhibit the release of ADH induced by nociceptive stimuli (O'Connor and Verney, 1945) and Ach (Duke and Pickford, 1951). The α-adrenoceptor blocking agents have been shown to block the antidiuretic response elicited by electrical stimulation of the hypothalamus or the central cut end of the ulnar nerve. All of these findings suggest that the SON and PVN may exhibit cholinergic and adrenergic interaction with respect to the control of ADH release.

In the present study, NA consistently produced an increased ADH release from the isolated SONH system. When administered into the carotid artery of conscious dogs, a dose-dependent relationship was obtained even in the presence of an elevated blood pressure. These findings are in agreement with that of Bhargava et al. (1974) who reported that NA, when injected into the cerebral ventricles of the dog, increased the ADH release. However, these results are in contrast to the findings of Schrier et al. (1974) who showed by indirect means (renal clearances) that NA infused into anesthetized dogs decreased ADH release. These investigators postulated that NA inhibits ADH release indirectly via raising the blood pressure. The reasons for the differences between their study and the present one

are not clear. However, it should be remembered that they did not measure ADH and that their animals were anesthetized while in the present study plasma ADH was measured directly and the experimental animals were conscious. The fact that NA produced an increase in ADH release in the in vitro system argues strongly that a direct adrenergic effect on ADH release exists. However, this is not to deny the fact that hemodynamic changes may alter ADH release and thus under certain circumstances prevent differentiation between primary and secondary effects.

Further support for the involvement of adrenergic receptors in the release of ADH comes from the studies with phentolamine. Phentolamine, when infused into the carotid artery of dogs, blunted the response to osmotic stimulation of the release of ADH. The location of these adrenergic receptors in the CNS is supported by the fact that phentolamine also inhibited the osmotically stimulated release of ADH in the isolated supraoptico neurohypophyseal preparation. These findings further support the concept that NA influences the release of ADH by a direct action on the supraoptic-neurohyphyseal system.

On the other hand, IP, a β-adrenoceptor stimulant drug had no effect on the release of ADH when studied in the isolated SONH system. Moreover, propranolol, a β-adrenoceptor blocking drug when

administered to conscious dogs, did not affect the osmotic stimulation of ADH release. Here again, these data are in agreement with that of Bhargava et al. (1972) who showed no changes with isoprenaline but in contrast with that of Schrier and his collaborators (1974) who observed an antidiuresis. However, just as with the α-adrenergic studies, the differences between the present studies and those of Schrier's group may be explained by the fact that 1) Schrier's group used anesthetized animals who experienced a significant fall in blood pressure when given IP, 2) no direct measurement of ADH was used in their studies. Thus, the present data would suggest that the adrenergic component of the SON and PVN is α-adrenergic only.